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(54) **MAPPING CYTOSINE MODIFICATIONS**

(71) Applicant: **New England Biolabs, Inc.**, Ipswich, MA (US)

(72) Inventors: **Shengxi Guan**, Stoneham, MA (US); **Nan Dai**, Gloucester, MA (US); **Zhenyu Zhu**, Beverly, MA (US); **Ivan R. Correa, Jr.**, Ipswich, MA (US); **Aine Quimby**, Newton, NH (US); **Janine Borgaro**, Beverly, MA (US)

(73) Assignee: **New England Biolabs, Inc.**, Ipswich, MA (US)

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This patent is subject to a terminal disclaimer.

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C07H 21/00	(2006.01)
C12P 19/18	(2006.01)
C12P 19/30	(2006.01)
C12P 19/34	(2006.01)
C12N 9/10	(2006.01)
C12N 9/22	(2006.01)
C07K 14/47	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 9/0069*** (2013.01); ***C07H 19/06*** (2013.01); ***C07H 21/00*** (2013.01); ***C07K 14/47*** (2013.01); ***C12N 9/1051*** (2013.01); ***C12N 9/22*** (2013.01); ***C12P 19/18*** (2013.01); ***C12P 19/30*** (2013.01); ***C12P 19/34*** (2013.01); ***C12Q 1/68*** (2013.01); ***C12Q 1/683*** (2013.01); ***C12Q 1/6806*** (2013.01); ***C12Q 1/6809*** (2013.01); ***C12Q 1/6827*** (2013.01); *C07K 2319/80* (2013.01); *C07K 2319/81* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2012/0301881	A1	11/2012	Zhu et al.
2013/0244237	A1	9/2013	Vaisvila et al.

FOREIGN PATENT DOCUMENTS

WO	WO20100037001		4/2010
WO	WO2011025819		3/2011
WO	WO 2011/091146	A1	7/2011
WO	WO2011091146		7/2011
WO	WO2011127136		10/2011

OTHER PUBLICATIONS

Adams et al. GeneBank Acession No. ABXKO1000045 (May 12, 2008).*
Borgaro, et al., Nucleic Acids Research, 41(5):1-9 (2013).
Booth, et al., Science, 336:934-937 (2012).
Jagdale, et al., Synthesis, 4:660-664 (2009).
Morales-Serna, et al., Synthesis, 9:1375-1382 (2011).
Falorni, et al., Tetrahedron Lett., 40: 4395-4396 (1999).
International Search Report for International Application No. PCT/US2013/068290 dated Mar. 11, 2014.
Szwagierczak, et al, Nucleic Acids Research, 19, e-181, 2010.
Li, et al., Chemistry and Biology, 11, 1, 107-119, 2004.
Seto, et al., Eur. J. Biochem, 259, 3, 770-775, 1999.
Song, et al., Nature Biotechnology, 29, 1, 68-72, 2011.
Yu, et al., Cell, 149, 6, 1368-1380, 2012.
Yu, et al., Nature Protocols, 7, 12, 2159-2170, 2012.
Borgaro et al Nucleic Acids Res. 2013 41: 4198-4206.
Wang et al Nucleic Acids Res. 2011 39: 9294-305.
Adams et al J. Bacteriol. 2008 190, 8053-8064.
Moréra et al J. Mol. Biol. 2001 311:569-77.
Ito et al Science. 2011 333: 1300-3.
Wu et al Genes Dev. 2011 25: 2436-52.
Williams et al EMBO Rep. 2012 13:28-35.
Hashimoto et al Nature. 2014 506: 391-5.
Saleh et al FASEB Journal 2014 28 Supplement 768.16.

* cited by examiner

Primary Examiner — Nashaat Nashed

(74) *Attorney, Agent, or Firm* — New England Biolabs, Inc; Harriet M. Strimpel

(57) **ABSTRACT**

Methods, compositions and kits for selectively altering and detecting modified cytosine residues are provided.

6 Claims, 8 Drawing Sheets

FIG. 1

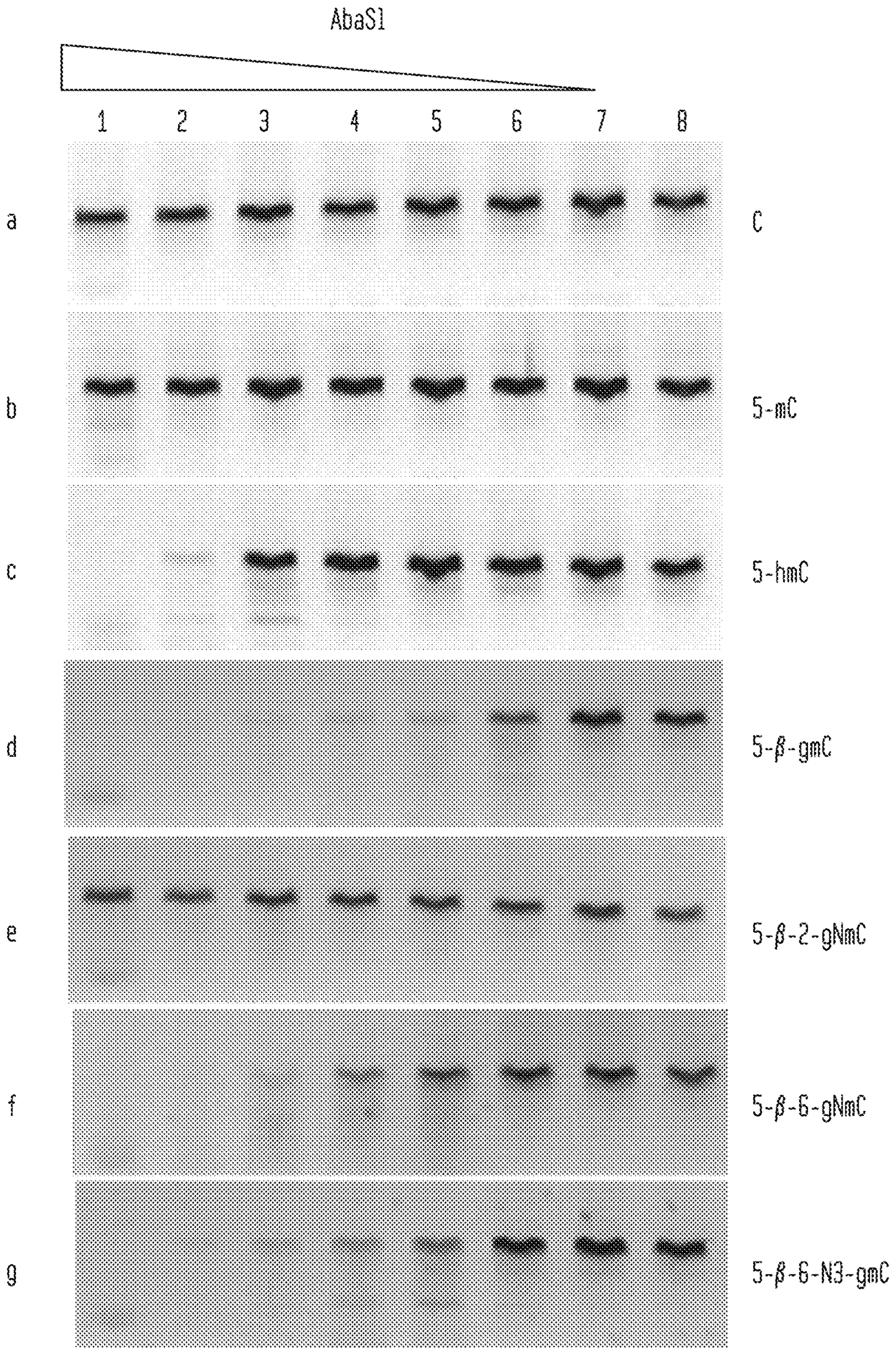
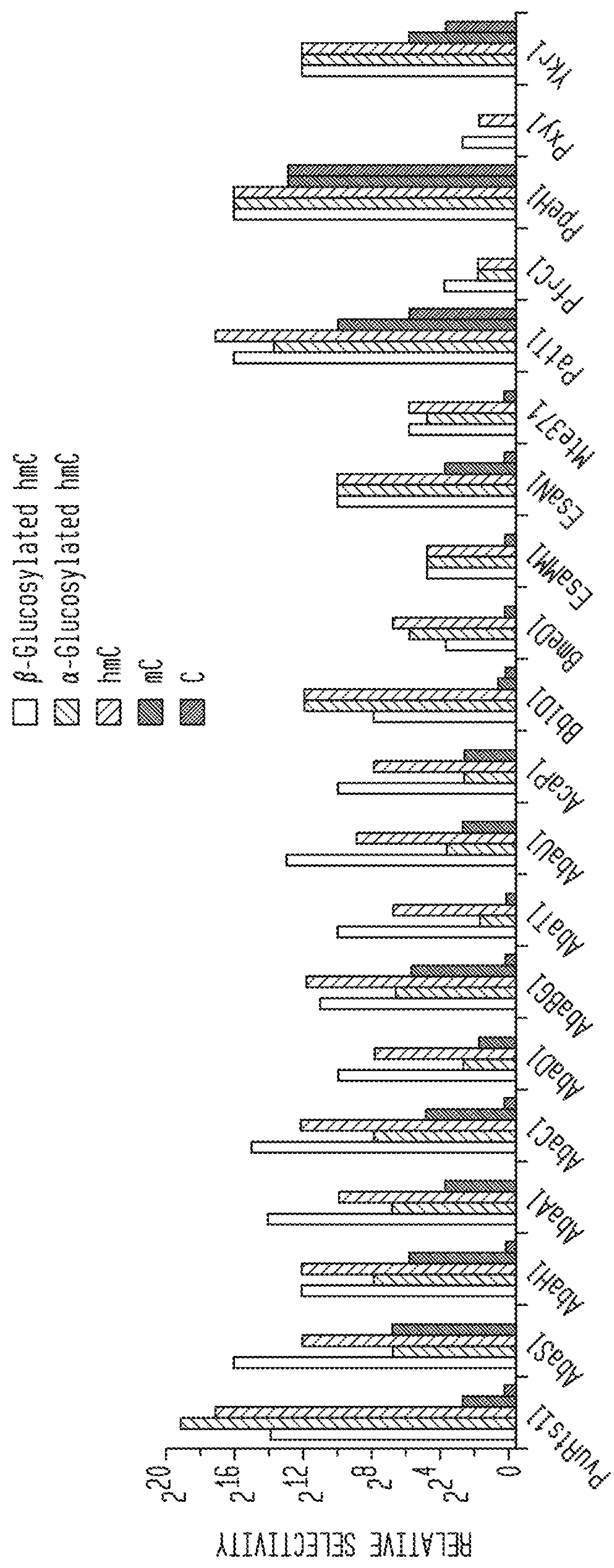

AbaSI
1
2
3
4
5
6
7
8
a
C
b
5-mC
c
5-hmC
d
5-β-gmC
e
5-β-2-gNmC
f
5-β-6-gNmC
g
5-β-6-N3-gmC

FIG. 2


β-Glucosylated hmC
α-Glucosylated hmC
hmC
mC
C
RELATIVE SELECTIVITY
2^{20}
2^{16}
2^{12}
2^{8}
2^{4}
0
PvuRts1I
AbaSI
AbaHI
AbaAI
AbaCI
AbaDI
AbaBGI
AbaTI
AbaUI
AcaPI
BbiDI
BmeDI
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EsaNI
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PfrCI
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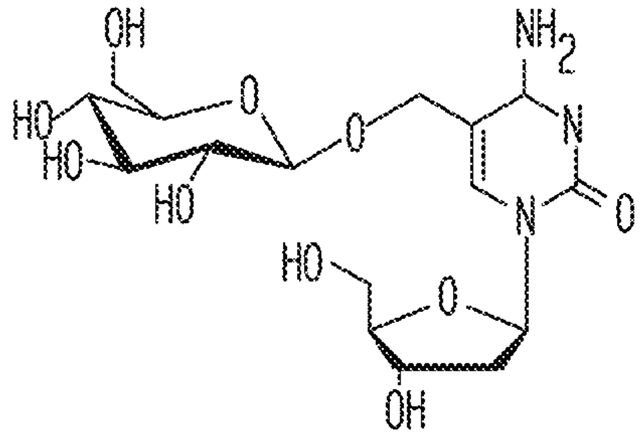
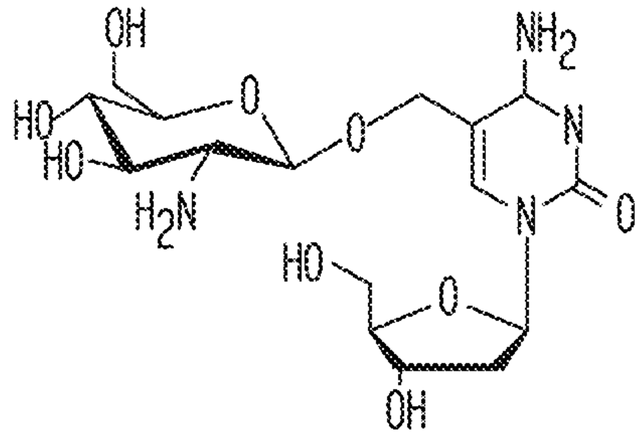
PxyI
YkrI

FIG. 3

T4-BGT



5-β-gmC



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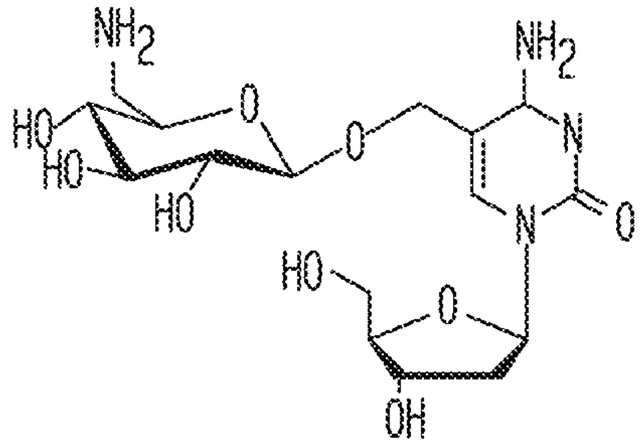
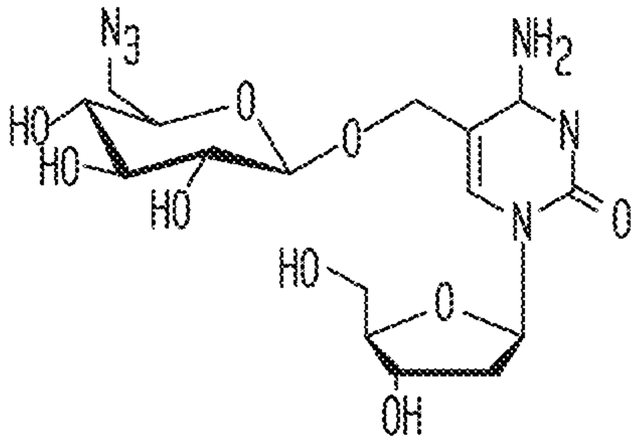
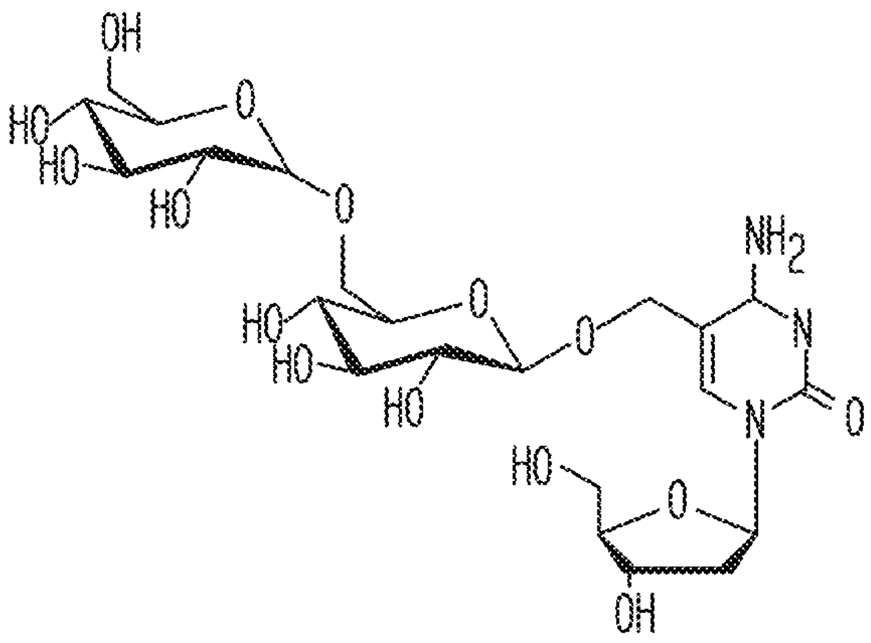


5-β-6-gNmC

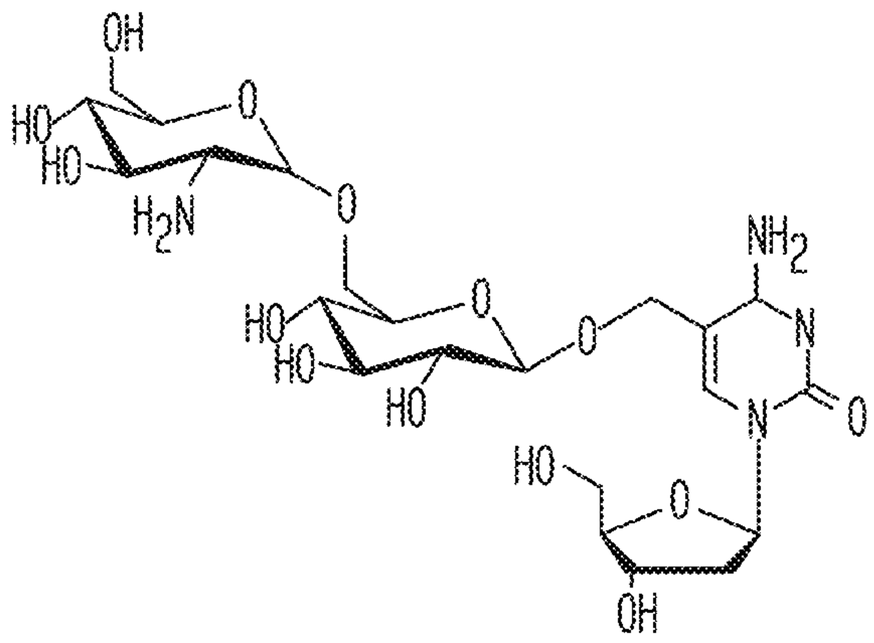


5-β-6-N_3-gmC

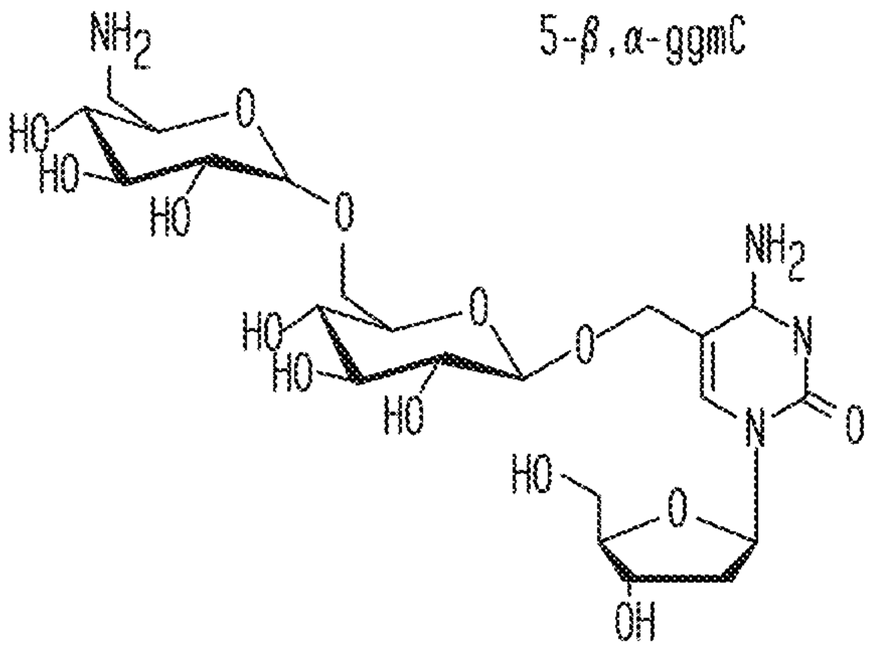
T4-BGT FOLLOWED BY T6-BGAGT



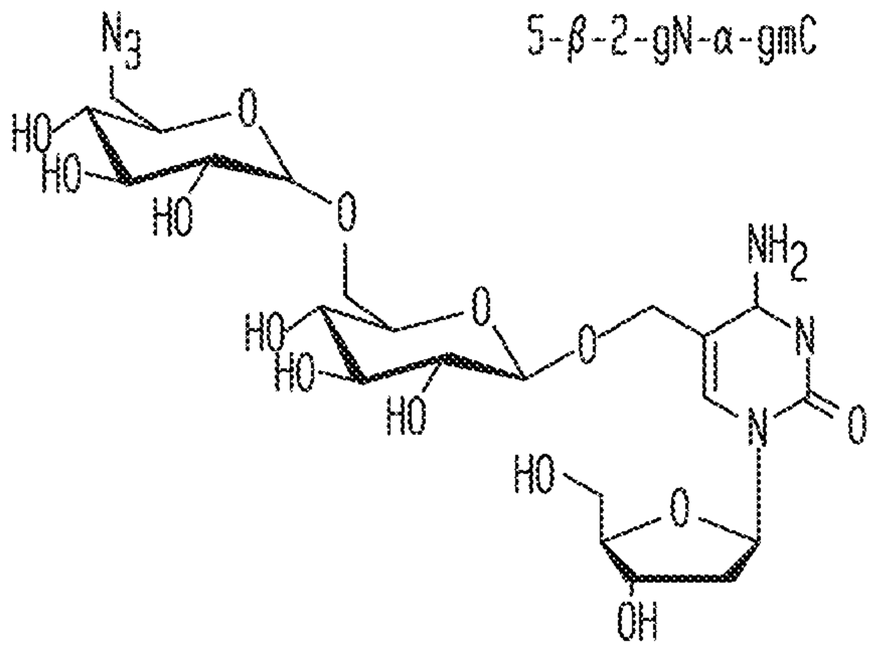
5-β,α-ggmC



5-β-2-gN-α-gmC



5-β-6-gN-α-gmC



5-β-6-N_3-α-gmC

FIG. 4

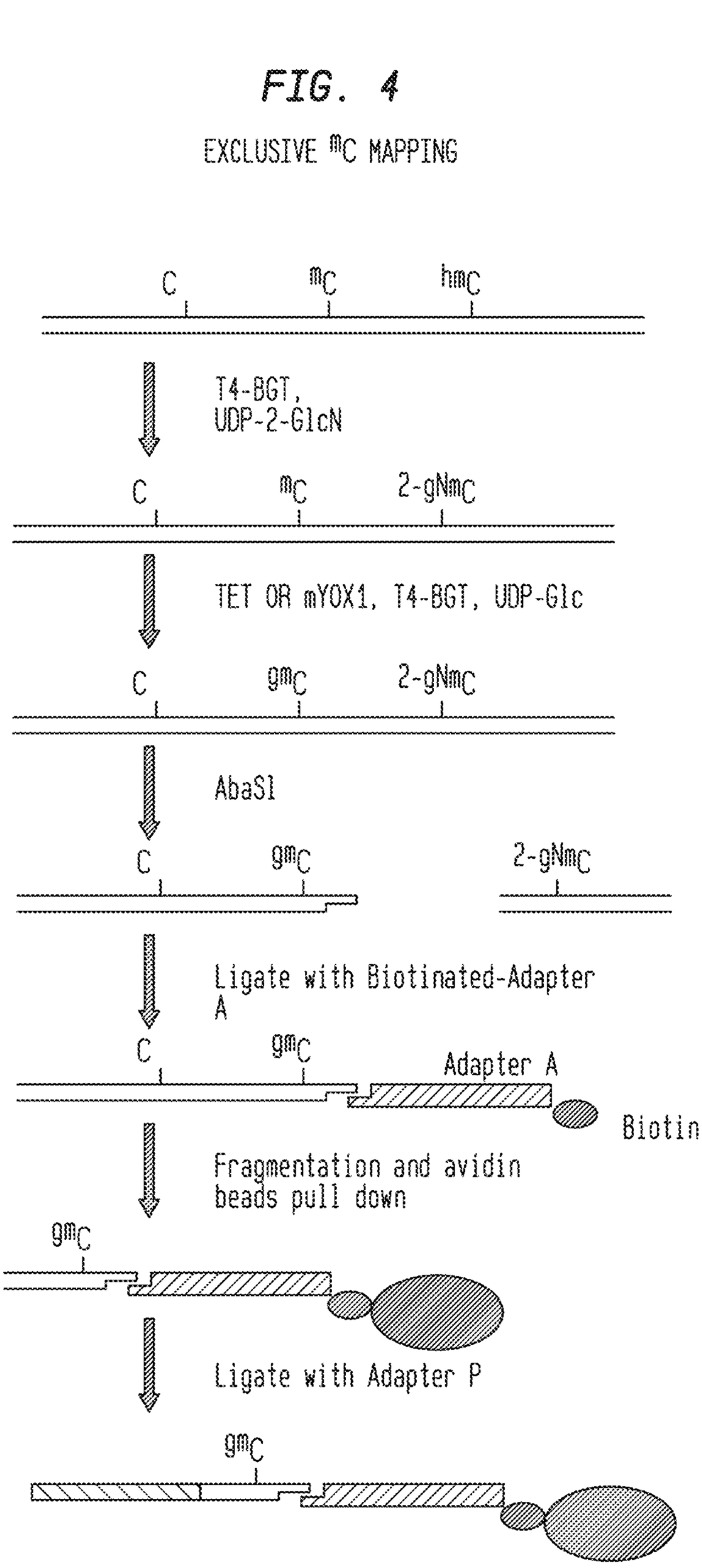

EXCLUSIVE mC MAPPING
C
mC
hmC
T4-BGT,
UDP-2-GlcN
C
mC
2-gNmC
TET OR mYOX1, T4-BGT, UDP-Glc
C
gmC
2-gNmC
AbaSI
C
gmC
2-gNmC
Ligate with Biotinated-Adapter
A
C
gmC
Adapter A
Biotin
Fragmentation and avidin
beads pull down
gmC
Ligate with Adapter P
gmC

FIG. 5A

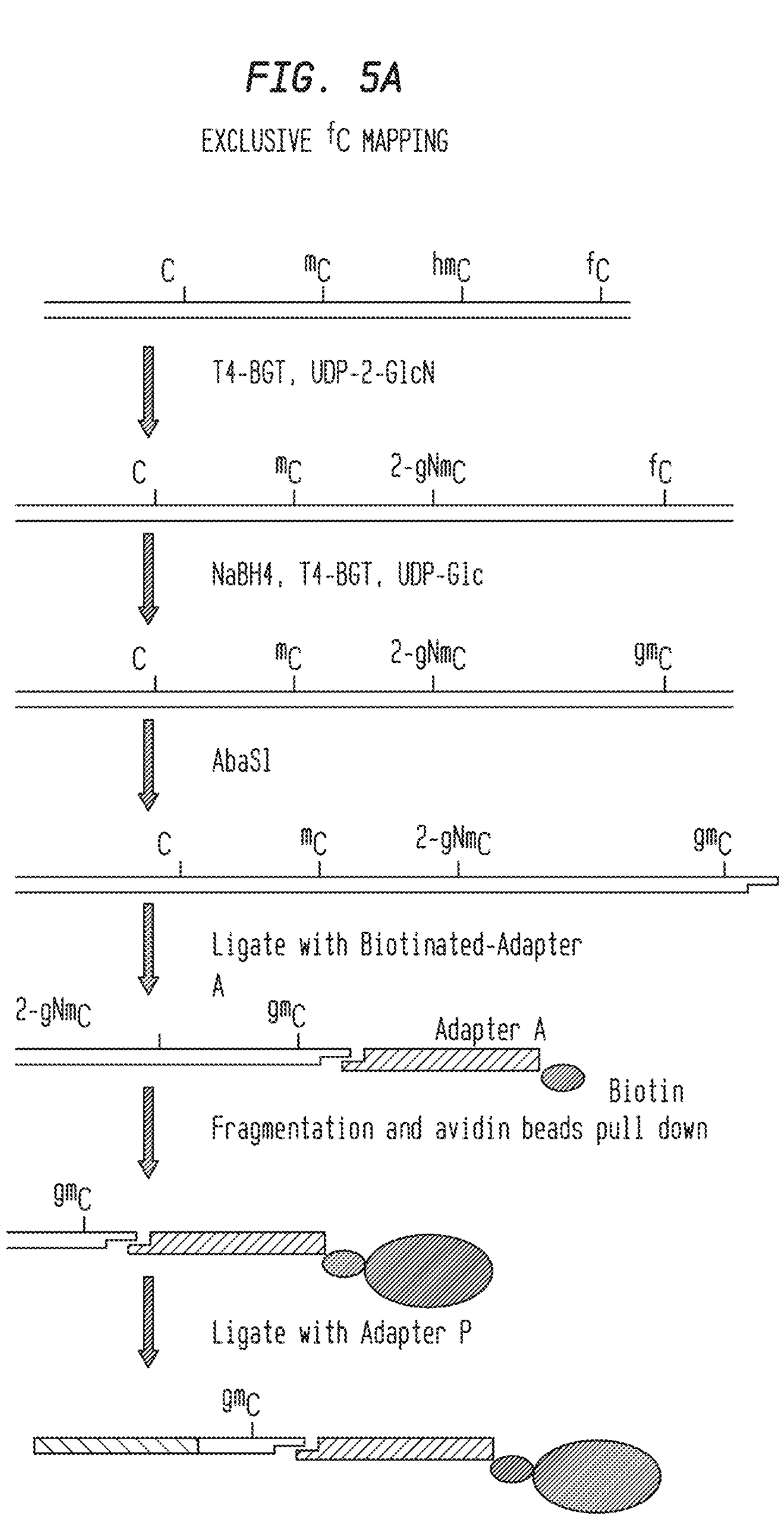

EXCLUSIVE fC MAPPING
C
mC
hmC
fC
T4-BGT, UDP-2-GlcN
C
mC
2-gNmC
fC
NaBH4, T4-BGT, UDP-Glc
C
mC
2-gNmC
gmC
AbaSI
C
mC
2-gNmC
gmC
Ligate with Biotinated-Adapter A
2-gNmC
gmC
Adapter A
Biotin
Fragmentation and avidin beads pull down
gmC
Ligate with Adapter P
gmC

FIG. 5B

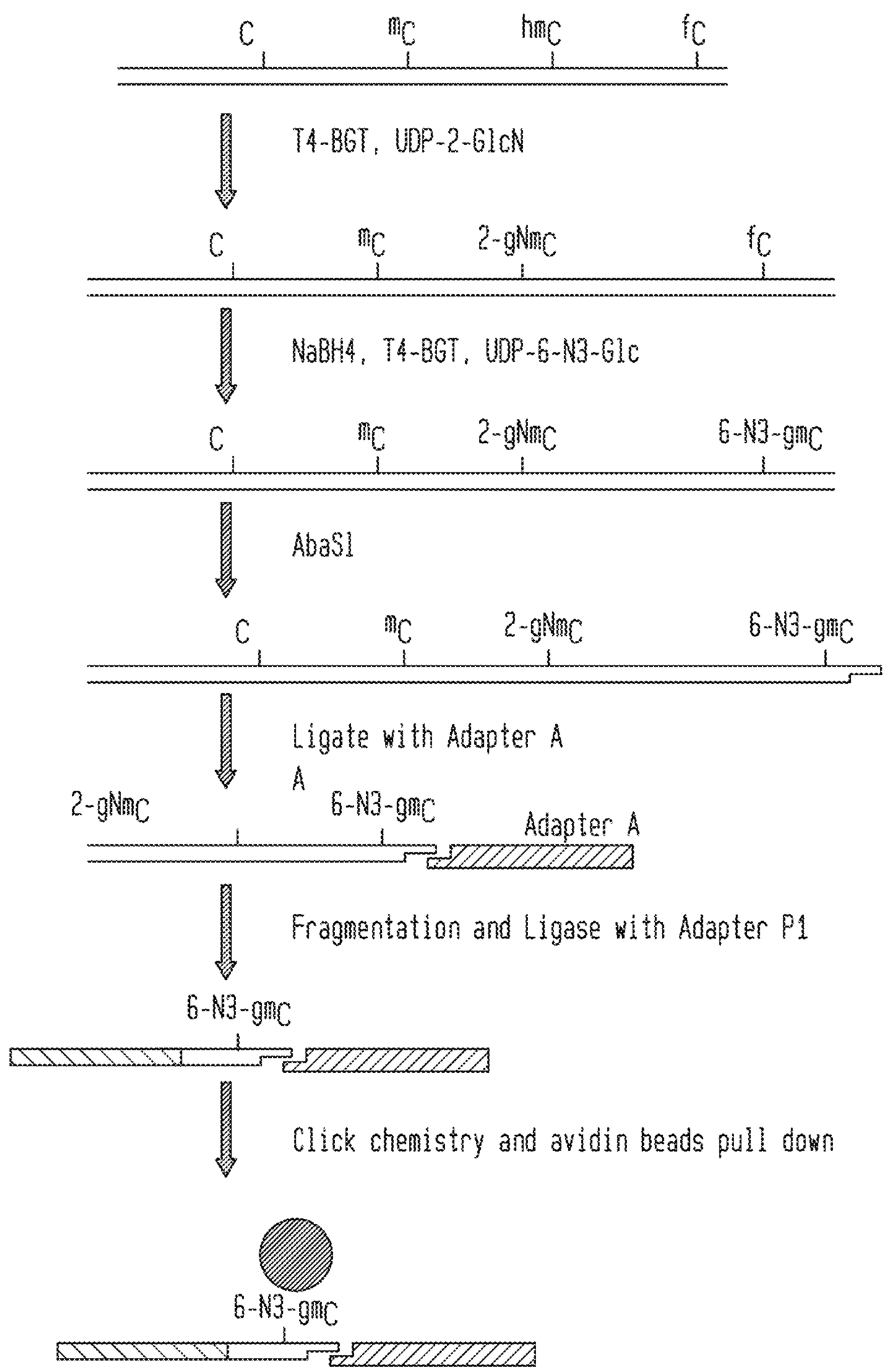

C
mC
hmC
fC
T4-BGT, UDP-2-GlcN
C
mC
2-gNmC
fC
NaBH4, T4-BGT, UDP-6-N3-Glc
C
mC
2-gNmC
6-N3-gmC
AbaSI
C
mC
2-gNmC
6-N3-gmC
Ligate with Adapter A
A
2-gNmC
6-N3-gmC
Adapter A
Fragmentation and Ligase with Adapter P1
6-N3-gmC
Click chemistry and avidin beads pull down
6-N3-gmC

FIG. 6

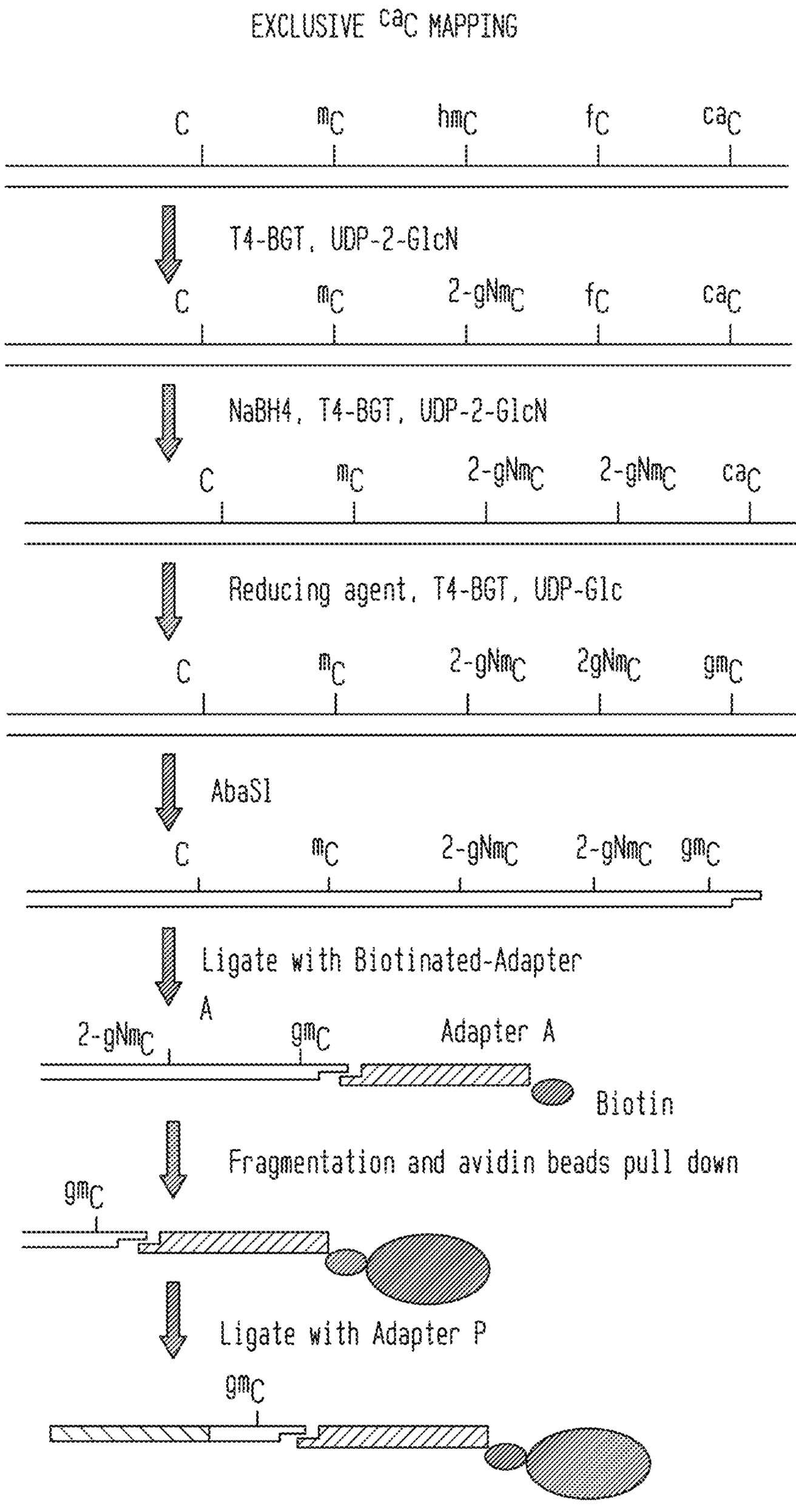
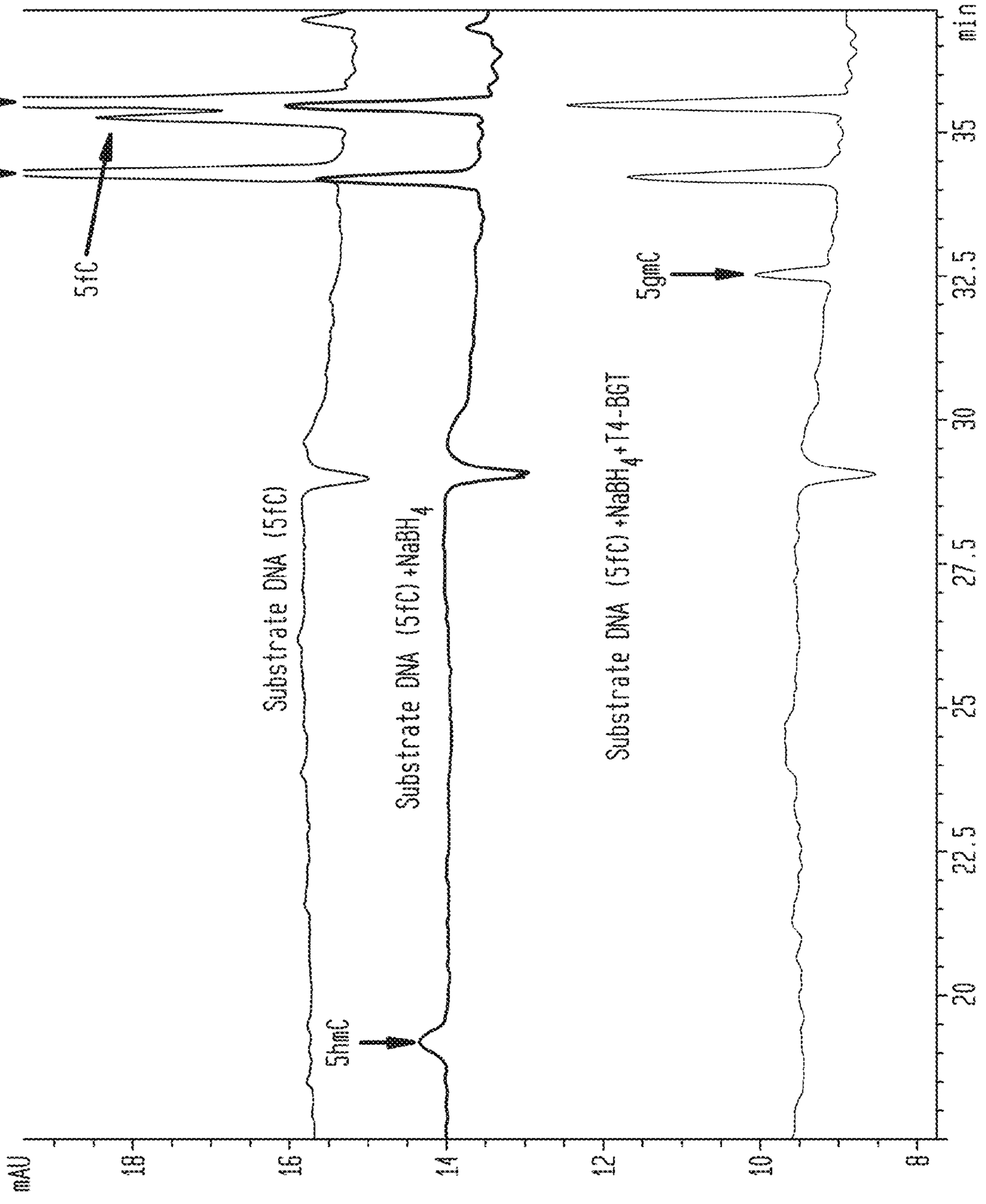

EXCLUSIVE caC MAPPING
C
mC
hmC
fC
caC
T4-BGT, UDP-2-GlcN
C
mC
2-gNmC
fC
caC
NaBH4, T4-BGT, UDP-2-GlcN
C
mC
2-gNmC
2-gNmC
caC
Reducing agent, T4-BGT, UDP-Glc
C
mC
2-gNmC
2gNmC
gmC
AbaSI
C
mC
2-gNmC
2-gNmC
gmC
Ligate with Biotinated-Adapter A
2-gNmC
gmC
Adapter A
Biotin
Fragmentation and avidin beads pull down
gmC
Ligate with Adapter P
gmC

FIG. 7


dT
dG
5fC
Substrate DNA (5fC)
Substrate DNA (5fC) +NaBH4
Substrate DNA (5fC) +NaBH4+T4-BGT
5gmC
5hmC
mAU
18
16
14
12
10
8
20
22.5
25
27.5
30
32.5
35
min

MAPPING CYTOSINE MODIFICATIONS

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of each of the following patent applications, each of which are hereby incorporated by reference into the present application: U.S. 61/611,295, filed Mar. 15, 2012; U.S. Application No. 61/722,968, filed Nov. 6, 2012; U.S. Application No. 61/723,427, filed Nov. 7, 2012; U.S. Application No. 61/724,041, filed Nov. 8, 2012; and U.S. application Ser. No. 13/804,804, filed Mar. 14, 2013. Also incorporated by reference in their entireties are the following applications filed on the same day as the present application: Ser. No. 13/827,087, "Compositions and Methods for Oxygenation of Nucleic Acids Containing 5-Methylpyrimidine"; and Ser. No. 13/827,885, "Methods and Compositions for Discrimination Between Cytosine and Modifications Thereof, and for Methylome Analysis."

GOVERNMENT RIGHTS

This invention was made with government support under GM096723 awarded by the National Institutes of Health. The government has certain rights in this invention.

BACKGROUND

5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxycytosine (5-caC) were recently identified in mammalian brain and embryonic stem cells as products of the oxidation of 5-methylcytosine (5-mC) by cytosine oxygenases. The biological functions of 5-hmC, 5-fC, and 5-caC are not completely understood; however, several lines of evidence suggest that 5-hmC is involved in epigenetic regulation and DNA demethylation. Iterative oxidation of 5-hmC by cytosine oxygenase enzymes yields 5-fC and 5-caC which are hypothesized to be intermediates in the DNA demethylation process. Several challenges are associated with the identification these biologically modified nucleobases in genomic DNA samples due to their low abundance and temporal fluctuation. Mapping and quantifying 5-mC, 5-hmC, 5-fC, and 5-caC at the DNA level is, therefore, important for unraveling their role in the dynamics of gene expression and regulation.

SUMMARY OF THE INVENTION

The present invention provides a variety of reagents, kits and methods for selectively altering and identifying modified nucleotides in a nucleic acid such as DNA. The modified nucleotides that can be identified include, for example, 5-mC, 5-hmC, 5-fC and 5-caC. The methods, reagents and kits of the present invention permit not merely the determination that a modified cytosine residue is present, but also permit the discrimination among or between different types of modified nucleotides, such as 5-mC and 5-hmC, or between 5-hmC and 5-fC. In this way, the invention can be used to elucidate the precise state of a modified nucleic acid, which may be a genome or genome fragment, for example.

In some embodiments, the discrimination among or between different oxidation states of a nucleotide is facilitated by alternately glucosylating or glucosaminylating various modified nucleotides. The glucosylated or glucosaminylated forms can be distinguished based on their performance in various assays, such as by their differential sensitivity to certain restriction endonucleases. In this way, alternately reacting the nucleic acid with a UDP-GlcN (Uridine diphosphate glucosamine or UDP-Glucosamine) substrate and a UDP-Glc (Uridine diphosphate glucose or UDP-Glucose) substrate, places off and on switches for endonuclease cleavage of a nucleic acid containing modified nucleotides. By detecting the presence of cleavage-sensitive sites, the modified nucleotides can be located and identified.

In the present invention, the term glucosylation (and any form of glucosylation such as "glucosylating" or "glucosylated") refers to the incorporation of a glucosyl moiety from UDP-Glc into the 5-hydroxy position of 5-hmC via the action of a glycosyltransferase to produce 5-gmC (5-glucosyloxymethylcytosine). Other names in common use for 5-gmC include glucosyl-5-hydroxymethyl-cytosine, glucosyl-5-hydroxymethylcytosine, glucosyl-oxy-5-methylcytosine, 5-glucosylhydroxymethylcytosine. In the present invention, the term glucosylation (and any form of glucosylation such as "glucosylating" or "glucosylated") may also refer to the incorporation of an azido modified glucosyl moiety from UDP-Azido-Glc (for example, UDP-6-Azido-Glc or UDP-6-N3-Glc) into the 5-hydroxy position of 5-hmC via the action of a glycosyltransferase to produce a N3-5-gmC (for example, 6-azide-glucosyl-5-hydroxymethylcytosine or 6-N3gmC). In the present invention, the term glucosaminylation (and any form of glucosaminylation such as "glucosaminylating" or "glucosaminylated") refers to the incorporation of a glucosaminyl moiety from UDP-GlcN into the 5-hydroxy position of 5-hmC via the action of a glycosyltransferase to produce 5-gNmC (5-glucosaminyloxymethylcytosine). Other names for 5-gNmC include glucosaminyl-5-hydroxymethyl-cytosine, glucosaminyl-5-hydroxymethylcytosine, 5-glucosylhydroxymethylcytosine, aminoglucosyl-5-hydroxymethyl-cytosine, aminoglucosyl-5-hydroxymethylcytosine, and 5-aminoglucosylhydroxymethylcytosine. If the glycosyltransferase is an inverting glycosyltransferase such as T4 β-glucosyltransferase (BGT or βGT or beta-GT) the product is formed with a beta glycosydic linkage (for example, 5-β-gmC, 5-β-6-N3gmC, 5-β-6-gNmC, and 5-β-2-gNmC). If the glycosyltransferase is a retaining glycosyltransferase such as T4 α-glucosyltransferase (AGT or αGT or alpha-GT) the product is formed with an alpha glycosydic linkage (for example, 5-α-gmC).

Specifically, for example, the invention permits the differentiation of 5-β-glucosyloxymethylcytosine (5-β-gmC) from 5-β-2-glucosaminyloxymethylcytosine (5-β-2-gNmC) in a nucleic acid, by reacting the nucleic acid with an endonuclease capable of cleaving a nucleic acid at a glucosylated nucleotide but not at an glucosaminylated nucleotide. One suitable endonuclease is AbaSI. Other useful endonucleases include AbaAI, AbaCI, AbaDI, AbaTI, AbaUI, AcaPI, and PxyI, or one of the ZZYZ proteins or its variants described in US Patent Application Publication No. 2012/0301881, the complete disclosure of which is hereby incorporated by reference. Accordingly, one of these endonucleases, or a polypeptide at least 70% (e.g. at least 75%, at least 80%, at least 82%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to one of those endonucleases or an active fragment thereof) can be used to selectively cleave a target nucleic acid. By controlling the conversion of glucosylation and glucosaminylation of various forms of naturally occurring modified nucleotides, the modified nucleotides can be located and identified in a manner distinguishing their original forms, such as methylcytosine, hydroxymethylcytosine, formylcytosine, and/or carboxycytosine.

Accordingly, in one aspect the invention provides methods for selectively altering modified nucleotides in a nucleic acid containing hydroxymethylated nucleotides and other modified nucleotides such as, 5-fC or 5-caC. The methods generally include reacting a first subset of the modified nucleotides (e.g. 5-hmC) in the nucleic acid with UDP-GlcN in the presence of a β-glycosyltransferase, such as T4-β-glucosyltransferase, to convert hydroxymethylated nucleotides in the nucleic acid to glucosaminylated nucleotides.

In some embodiments, the methods include the subsequent step of reacting the nucleic acid with a reducing agent, such as $NaBH_4$, and a UDP derivative, such as UDP-Glc or UDP-azido-glucose, to convert a second subset of nucleotides in the nucleic acid to glucosylated or azidoglucosylated nucleotides. The reducing agent promotes the reduction of a nucleotide in a higher oxidation state, such as 5-fC or 5-caC, to a hydroxymethylated nucleotide, which is then glucosylated or azidoglucosylated in a reaction typically catalyzed by a glycosyltransferase such as an α-glycosyltransferase or a β-glycosyltransferase. The glycosyltransferase may or may not be the same glycosyltransferase used to catalyze the previous conversion of hydroxymethylated nucleotides to glucosaminylated nucleotides. The method can optionally differentiate pre-existing 5-hmC in a nucleic acid from newly formed as a result of the reduction of 5-fC or 5-caC, for example. When a UDP-azido-glucose such as UDP-6-azido-glucose is used, 5-hmC in the nucleic acid can be converted to a β-6-azide-glucosyl-5-hydroxymethylcytosine in the presence of a β-glycosyltransferase; Further derivatization of the azido moiety via azide-alkyne Huisgen cycloaddition using a copper(I) catalyst ("click chemistry") or via copper-free variants (for example, using strained cyclooctyne derivatives) can then optionally be used to label the nucleotide, such as with a biotin label, permitting the subsequent use of avidin to selectively isolate the labeled nucleotide.

In other embodiments, following the conversion of hydroxymethylated nucleotides in the nucleic acid to glucosaminylated nucleotides, the nucleic acid is reacted with an oxidizing agent, a UDP-Glc derivative, and a glycosyltransferase to convert a second subset of modified nucleotides in the nucleic acid to glucosylated nucleotides. Suitable oxidizing agents include those capable of oxidizing 5-mC to 5-hmC, such as mYOX1, a TET enzyme, or an inorganic oxidizing agent such as $KRuO_4$. The reaction with UDP-Glc is typically conducted in the presence of a glycosyltransferase such as an α-glycosyltransferase or a β-glycosyltransferase. These methods can, for example, differentiate pre-existing 5-hmC in a nucleic acid from 5-hmC generated from a 5-mC precursor.

In any of these methods, whether incorporating a reducing agent or an oxidizing agent, an endonuclease can be used to characterize the reaction products. Typically, the endonuclease is specific for the glucosylated (or azidoglucosylated) nucleotides, and not for glucosaminylated nucleotides, i.e., the endonuclease has a higher enzymatic activity for a glucosylated or azidoglucosylated nucleic acid that it has for the same nucleic acid with an glucosaminylated nucleotide at the same position(s). Endonucleases that may be used include, for example, AbaSI, AbaAI, AbaCI, AbaDI, AbaTI, AbaUI, AcaPI, and/or PxyI. An adapter molecule can then be ligated to the cleaved end of the endonuclease reaction product, facilitating the subsequent purification, amplification or sequencing of the nucleic acid.

Accordingly, in one embodiment the invention provides a method for differentiating a 5-mC from 5-hmC in a genome or genome fragment. The genome or genome fragment may, for example, be mammalian in origin, such as a human genome or genome fragment. The method includes reacting the isolated genome or genome fragment containing 5-mC and 5-hmC with (i) UDP-GlcN in the presence of a glycosyltransferase catalyzing transfer of glucosamine to the 5-hmC; (ii) oxygenating any existing 5-mC residues to 5-hmC by the action of TET or mYOX1; (iii) reacting the newly created 5-hmC sites with UDP-Glc in the presence of a glycosyltransferase catalyzing transfer of glucose to the 5-hmC; (iv) cleaving the glucosylated template with a modification-dependent endonuclease that recognizes at least one of the modified nucleotides; and (v) differentiating the original 5-mC from the 5-hmC by an altered cleavage pattern.

In this embodiment, the oxygenation of any existing 5-mC residues to 5-hmC can be done in the presence of UDP-Glc and a glycosyltransferase to catalyze the transfer of glucose to 5-hmC as it is being formed from 5-mC. Alternatively, reaction conditions such as pH of the oxygenation reaction can be selected to optimize the yield of 5-hmC. See, for example, Ser. No. 13/827,087, "Compositions and Methods for Oxygenation of Nucleic Acids Containing 5-Methylpyrimidine," filed on the same date as the present application and hereby incorporated by reference in its entirety.

In another embodiment, the invention provides a method for differentiating a 5-mC from one or more of its oxidation products in a genome or genome fragment containing 5-mC and 5-hmC. The method includes reacting the isolated genome or genome fragment with UDP-2-glucosamine in the presence of a β-glycosyltransferase (BGT) catalyzing the transfer of 2-glucosamine to the 5-hmC; reacting the isolated genome or genome fragment with mYOX1 or TET or a chemical oxidizing agent and optionally with a reducing agent; cleaving the template with a modification dependent endonuclease that is capable of selectively cleaving a 5-hmC and not a 5-glucosaminated hydroxymethylcytosine; and differentiating the 5-mC from one or more of its oxidation products by an altered cleavage pattern.

The invention also provides preparations useful for converting methylcytosine or an oxidized nucleotide, such as 5-fC or 5-caC, or to a glucosylated nucleotide. The preparations include a reducing agent, such as $NaBH_4$, or an oxidizing agent, such as mYOX1, a TET enzyme, or an inorganic oxidizing agent such as $KRuO_4$; a glycosyltransferase, such as an α-glycosyltransferase or a β-glycosyltransferase; a UDP-GlcN or a UDP derivative, such as UDP-Glc or UDP-azido-glucose.

The invention also provides preparations useful for modifying and selectively cleaving nucleic acids. The preparations include a glycosyltransferase and an endonuclease having an amino acid sequence at least 95% identical to an enzyme such as AbaSI, AbaAI, AbaCI, AbaDI, AbaTI, AbaUI, AcaPI, and/or PxyI. The preparations also include (a) UDP-Glc and an oxidizing agent, or (b) UDP-GlcN. Where UDP-GlcN is included, the glycosyltransferase may be a BGT to catalyze the transfer of glucose to hydroxymethylated pyrimidine residues. Where UDP-Glc and an oxidizing agent are included, the oxidizing agent, which may be a methylcytosine oxygenase such as mYOX1 or a TET enzyme or may be an inorganic oxidizing agent such as $KRuO_4$, promotes the conversion of methylcytosine residues in a nucleic acid to hydroxymethylcytosine, which can be glucosylated by the combination of UDP-Glc and the glycosyltransferase, and can be recognized by the endonuclease.

The invention also provides kits useful for making these preparations and practicing these methods. For example, kits for modifying formylcytosine or carboxycytosine residues in a nucleic acid can include a reducing agent, such as sodium borohydride, permitting the reduction of formylcytosine or

carboxycytosine residues to hydroxymethylcytosine; a glycosyltransferase (such as a β-glycosyltransferase); and a UDP derivative, such as UDP-Glc and/or UDP-azidoglucose (such as UDP-6-azidoglucose), permitting the transfer of a sugar or modified sugar to the hydroxymethylcytosine. These kits may also include an oxidizing agent (e.g., a methylcytosine oxygenase such as mYOX1 or a TET enzyme or an inorganic oxidizing agent such as $KRuO_4$) to promote the conversion of methylcytosine residues in a nucleic acid to hydroxymethylcytosine; UDP-GlcN; a restriction endonuclease (e.g., having an amino acid sequence at least 95% identical to AbaSI, AbaAI, AbaCI, AbaDI, AbaTI, AbaUI, AcaPI, and/or PxyI); or any combination of the above.

Kits for selectively modulating the susceptibility of modified nucleic acid residues to cleavage can include UDP-Glc; UDP-GlcN; a β-glycosyltransferase; and a reducing (e.g. sodium borohydride) or oxidizing agent (e.g., a methylcytosine oxygenase such as mYOX1 or a TET enzyme or an inorganic oxidizing agent such as $KRuO_4$).

Kits useful for modifying and selectively cleaving nucleic acids can include, for example, a glycosyltransferase and an endonuclease having an amino acid sequence at least 95% identical to an enzyme such as AbaSI, AbaAI, AbaCI, AbaDI, AbaTI, AbaUI, AcaPI, and/or PxyI. These kits can also include (a) UDP-Glc and an oxidizing agent, and/or (b) UDP-GlcN.

Some embodiments of the kits or preparations of the present invention include an optional nucleic acid, such as a nucleic acid that is to be modified, that is undergoing modification, and/or a control nucleic acid. Accordingly, a nucleic acid, if present, may include 5-hmC, 5-gmC, 5-gNmC, 5-fC, 5-caC, 5-mC, or any combination of the above.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows that AbaSI recognizes 5-(β-glucosyloxymethyl)cytosine (5-β-gmC) but not 5-(β-2-glucosaminyloxymethyl)cytosine (5-β-2-gNmC) with high specificity as compared to 5-mC and C. The different forms of cytosine modification (panels a-g) were digested by a 10-fold serial dilution of AbaSI enzyme (lane 1 to lane 7, lane 8 is undigested control).

FIG. **2** depicts the relative selectivity of a variety of endonucleases for nucleic acid substrates containing 5-β-gmC ("β-glucosylated hmC"), 5-α-gmC ("α-glucosylated hmC"), 5-hydroxymethylcytosine ("hmC"), 5-methylcytosine ("mC"), or unmodified cytosine ("C").

FIG. **3** depicts selected cytosine modifications that can be achieved through the addition of glucose, modified glucose, or glucosamine residues by T4-BGT alone or in combination with β-glucosyl-α-glycosyltransferase from bacteriophage T6 ("T6-BGAGT").

FIG. **4** depicts one example of an assay for detecting and/or mapping 5-mC.

FIG. **5** depicts two examples of assays for detecting and/or mapping 5-fC. The assay in FIG. **5**A includes a step involving glucosylating hydroxymethylcytosine residues newly-formed from the reduction of 5-fC. The assay in FIG. **5**B includes a step involving azidoglucosylating hydroxymethylcytosine residues newly-formed from the reduction of 5-fC.

FIG. **6** depicts an example of an assay for detecting and/or mapping 5-caC.

FIG. **7** depicts LC-MS analyses of a nucleic acid containing 5-fC (top trace) and the same nucleic acid after treatment with 100 mM sodium borohydride (middle trace) or with sodium borohydride, UDP-Glc, and T4-BGT (bottom trace).

DETAILED DESCRIPTION OF EMBODIMENTS

Endonucleases have been identified from bacteria and more will undoubtedly be discovered using routine BLAST searches based on the present disclosure that are capable of preferentially cleaving 5-β-gmC compared to C, 5-mC and 5-β-2-gNmC. In one example, the ZZYZ family members for example AbaSI (see for example WO 2011/091146), isoschizomers and mutants thereof preferentially cleave 5-β-gmC compared to C, 5-mC and 5-β-2-gNmC. For example, AbaSI has cleavage activity at 5-β-gmC that is 500 fold greater than 5-mC and 5-α-gmC. 5-β-gmC is the product of BGT mediated transfer of glucose from UDP-Glc to 5-hmC.

The specificity of AbaSI is demonstrated in the data shown in FIG. **1**. AbaSI cleaves recognizes nucleic acids containing certain cytosine modifications, cleaving them a short distance from those modifications. Ten-fold serial dilutions of AbaSI were combined with a PCR product containing cytosine ("C," panel a), 5-methylcytosine ("5-mC," panel B), 5-hydroxymethylcytosine ("5-hmC," panel C), 5-(β-glucosyloxymethyl) cytosine ("5-β-gmC," panel D), 5-(β-2-glucosaminyloxymethyl)cytosine ("5-(β-2-gNmC," panel E), 5-(β-6-glucosaminyloxymethyl)cytosine ("5-β-6-gNmC," panel F), or 5-(β-6-azidoglucosyloxymethyl)cytosine ("5-β-6-N3gmC," panel G). The PCR products containing 5-β-gmC, 5-β-2-gNmC, 5-β-6-gNmC, or 5-β-6-N3gmC were generated by treatment of 5-hmC PCR DNA (panel c) with T4-β-glucosyltransferase (BGT) and the corresponding UDP-sugar (uridine diphospho-glucose (UDP-Glc), UDP-2-glucosamine (UDP-2-GlcN), UDP-6-glucosamine (UDP-6-GlcN), and UDP-6-N3-Glc, respectively). The results show that AbaSI does not digest C and 5-mC even at high concentrations (panels a and b) whereas AbaSI can digest 5-β-gmC even at very low concentrations (panel d). With respect to the three glucosylated or glucosaminated cytosines, AbaSI can digest 5-β-6-gNmC and 5-β-6-N3gmC (panels f and g) more efficiently than it digests 5-hmC (although less efficiently than it digests 5-β-gmC. In contrast, it does not digest 5-β-2-gNmC (panel e).

Other endonucleases that differentiate between various forms of modified cytosine are also available, such as other ZZYZ family members described in WO 2011/091146 and enzymes described in Borgaro et al. (2013) "Characterization of the 5-hydroxymethylcytosine-specific DNA restriction endonucleases," *Nucleic Acids Research*, doi: 10.1093/nar/gkt102, the entire disclosures of each of which are incorporated herein by reference. Several endonucleases that discriminate among various cytosine modifications are described in FIG. **2**. For example, PvuRts1I cleaves nucleic acids containing hydroxymethylcytosine or glucosyloxymethylcytosine far more efficiently than it cleaves nucleic acids containing only methylcytosine or unmodified cytosine. AbaSI, as described in the preceding paragraph, cleaves nucleic acids containing 5-β-gmC more efficiently than it cleaves nucleic acids containing 5-hmC, which are nevertheless cleaved more efficiently than those nucleic acids containing only 5-mC or unmodified cytosine. Like AbaSI, AbaAI, AbaCI, AbaDI, AbaTI, AbaUI, AcaPI, and PxyI all demonstrate increased specificity for nucleic acids containing 5-β-gmC compared to nucleic acids containing only 5-hmC. Accordingly, for any of these enzymes β-glucosylation of hydroxymethylcytosine can be used as an "on switch" to promote cleavage near those positions, whereas β-glucosylation could be used as an "off switch" for enzymes such as

PvuRts1I, AbaBGI, BbiDI, BmeDI, or PatTI. On the other hand, α-glucosylation of 5-hmC generates a more efficient substrate for PvuRts1I, and could be used as an "on switch" to target cleavage events to those locations in a nucleic acid.

It is expected that the use of endonucleases with preferential specificity for a specific modified nucleotide over other modified and unmodified nucleotides can be detected using the method described herein for 5-mC, 5-hmC, 5-fC, and 5-caC. In combination with the cofactor UDP-Glc, this system enables sequencing different epigenetic states of 5-mC and greatly enhances the ability to determine the epigenetic modification at a single base resolution level.

An embodiment of the method relies on modifying the non-target modified base (for example, 5-hmC) chemically and/or enzymatically in such a way that its reactivity to a endonuclease is completely or partially blocked, and then chemically or enzymatically reacting the target modified base to convert it into a newly formed 5-hmC (e.g., with a reducing or oxidant agent) which can be then be reacted with a glycosyltransferase to form 5-gmC which in turn can be cleaved by an endonuclease that recognizes 5-gmC preferentially in a positive identification.

By the appropriate choice of the substrate for modifying the non-target and target modified bases, this invention provides an on-off switch assay which is determined by enzyme specificity. For example, if in the first step UDP-2-GlcN is used to label pre-existing 5-hmC sites (resulting in an off-signal for AbaSI cleavage), and in the second step the sample is treated with a methylpyrimidine oxygenase (mYOX), BGT and UDP-Glc, an "on-signal" for AbaSI cleavage will be generated exclusively for 5-mC sites which underwent oxidation by mYOX. If UDP-Glc is used to label pre-existing 5-hmC sites (on-signal for AbaSI cleavage), and the sample is treated with an mYOX, BGT and UDP-2-GlcN, then an "off-signal" will be generated for all 5-mC sites which were converted into 5-hmC by mYOX-mediated oxidation.

The 5-mC may be chemically or enzymatically converted to 5-hmC by reacting the 5-mC with mYOX1 (see for example, "nMCO1" described in U.S. Provisional Application No. 61/723,427), TET enzymes or chemical oxidizing agents. Similarly, oxidation of 5-mC to 5-hmC to 5-fc to 5-CaC can be achieved by chemical or enzymatic oxidation using mYOX1 or TET. Specific chemical oxidation of 5-hmC to 5-fC in synthetic nucleotide oligomer single strand (ssDNA) containing 5-hmC can be achieved with potassium perruthenate, KRuO4 (Booth, et al. *Science,* 336:934-937 (2012)). KRuO4 can oxidize 5-hmC in double-stranded DNA (dsDNA), with an initial denaturing step before the addition of the oxidant, resulting in quantitative conversion of 5-hmC to 5-fC. Other oxidants known in art, such as Osmium (VIII)-based oxidants, Cerium (IV)-based oxidants, and Chromium (VI)-based oxidants may be used for the oxidation of 5-hmC to 5-fC.

A variety of sugars and modified sugars can be used to after the propensity of a 5-hmC to trigger an endonuclease-mediated cleavage event. Some of these sugars and modified sugars are depicted in FIG. **3**. For example, T4-BGT can be used to add glucose, 2-glucosamine, 6-glucosamine, or 6-azido-glucose to 5-hmC. T4-BGT can also be used in combination with a β-glucosyl-α-glycosyltransferase, such as the one from bacteriophage T6, to generate disaccharidyl cytosine modifications as shown in FIG. **3**. By controlling the modifications made to a particular nucleobase, the properties of that nucleobase can be changed in a manner facilitating its discrimination, whether through changes in its reactivity with an endonuclease; changes in the kinetics of synthesis of a complementary nucleic acid; or directly measured changes in size, shape, or charge density.

The reduction of 5-fC and its conversion to 5-β-gmC was demonstrated in the experiment depicted in FIG. **7**. Specifically, FIG. **7** is an LC-MS analysis of a nucleic acid originally including 5-fC (top trace). In the presence of sodium borohydride ($NaBH_4$), the 5fC is converted to 5-hmC (middle trace). When UDP-Glc and a BGT such as T4-BGT were also provided, the 5-fC was converted all the way to 5-β-gmC, confirming that the modified forms of cytosine can be interconverted, facilitating their subsequent detection and discrimination.

In one embodiment the method comprises one or more of the following steps;

(a) Genomic DNA is treated with BGT and UDP-2-GlcN, so that all 5-hmC residues are converted to 5-β-2-gNmC.

(b) (i) The resulting DNA is treated with mYOX1, a Tet enzyme or a chemical oxidant agent (converts 5-mC to 5-hmC), BGT and UDP-Glc, so that all existing 5-mC residues are converted to 5-β-gmC; or

(ii) The resulting DNA is treated with a reducing agent (e.g., NaBH4, converts 5-fC to 5-hmC), BGT and UDP-Glc to generate 5-β-gmC; or

(iii) The resulting DNA is treated with a reducing agent (e.g., NaBH4, converts 5-fC to 5-hmC), BGT and UDP-2-GlcN, so that all 5-fC residues are converted to 5-β-2-gNmC. Then, the resulting DNA is treated with a second reducing agent (a different reducing agent or the same reducing agent but in the presence of certain additives that converts 5-caC to 5-hmC), BGT, and UDP-Glc to generate 5-β-gmC.

(c) (i) The DNA is digested with a 5-β-gmC-dependent endonuclease such as AbaSI, which cleaved at a fixed distance from 5-β-gmC and left a sticky end (2-base 3'-overhang). Since the endonuclease does not recognize C or 5-β-2-gNmC no cleavage associated with these sites occurs. The only sticky ends created are those resulting from 5-β-gmC residues, which in turn are exclusively associated to 5-mC sites; or

(ii) The DNA is digested with endonuclease, which cleaves 5-β-gmC exclusively associated to 5-fCs sites, but not 5-β-2-gNmC,

(iii) the DNA is digested with endonuclease, which cleaves 5-β-gmC exclusively associated to CaC sites, but not 5-β-2-gNmC, 5-mC, or C, leaving a sticky end (2-base 3'-overhang).

(d) A first adaptor (e.g., biotinylated adaptor A) is then ligated onto the cleaved ends.

(e) The ligated DNA is then subjected to random fragmentation to about 200 bp.

(f) Beads may be used to pull out the fragments with the ligated adaptor. For example, avidin beads may be used to pull out the biotin labeled adaptor (adaptor A). A person of ordinary skill in the art will recognize other affinity systems and immobilization matrices that can be used in place of biotin/avidin beads.

(g) After polishing the ends, adaptor P is then ligated onto the DNA fragments on the beads.

(h) The adaptor-specific PCR and the adapter ligated DNA enters the library preparation pipeline for specific sequencing platform where the end-sequencing is done from the adaptor A.

Reducing agents and conditions can be used herein that can convert the carboxylic acids into alcohols, e.g., $NaBH_4$, $CoCl_2$, i-Pr_2NH, $EtOH/H_2O$ (Jagdale, et al. *Synthesis,* 660-664 (2009)); EDC, HOBt, $NaBH_4$, THF/H_2O (Morales-

Serna, et al. *Synthesis,* 1375-1382 (2011)); and cyanuric chloride, $NaBH_4$, NMM/H_2O (Falorni, et al. *Tetrahedron Lett.,* 4395-4396 (1999)) as well as other reducing agents known to a person of ordinary skilled in the art.

Indeed, many water-soluble metal or metalloid hydrides are able to reduce aldehydes and/or carboxylic acids to alcohols. Examples of such reducing agents are sodium borohydride and related compounds where from 1 to 3 of the hydrogens are replaced by other moieties, such as cyano and alkoxy containing up to about 5 carbon atoms. Examples of substituted borohydrides, all of which are sodium, potassium, or lithium salts, include cyanoborohydride, dicyanoborohydride, methoxyborohydride, dimethoxyborohydride, trimethoxyborohydride, ethoxyborohydride, diethoxyborohydride, triethoxyborohydride, propoxyborohydride, dipropoxyborohydride, tripropoxyborohydride, butoxyborohydride, dibutoxyborohydride, tributoxyborohydride, and so forth. Examples of other water-soluble metal hydrides include lithium borohydride, potassium borohydride, zinc borohydride, aluminum borohydride, zirconium borohydride, beryllium borohydride, and sodium bis(2-methoxyethoxy)aluminium hydride. Sodium borohydride can also be used in combination with a metal halide, such as cobalt(II), nickel(II), copper(II), zinc(II), cadmium (II), calcium (II), magnesium(II), aluminum(III), titanium (IV), hafnium(IV), or rhodium(III), each of which can be provided as a chloride, bromide, iodide, or fluoride salt. Alternatively, sodium borohydride can be used in combination with iodine, bromine, boron trifluoride diethyl etherate, trifluoroacetic acid, catechol-trifluoroacetic acid, sulfuric acid, or diglyme. Particular reducing strategies include the combination of potassium borohydride with lithium chloride, zinc chloride, magnesium chloride, or hafnium chloride; or the combination of lithium borohydride and chlorotrimethylsilane. Other reducing strategies include the use of borane, borane dimethyl sulfide complex, borane tetrahydrofuran complex, borane-ammonia complex, borane morpholine complex, borane dimethylamine complex, borane trimethylamine complex, borane N,N-diisopropylethylamine complex, borane pyridine complex, 2-picoline borane complex, borane 4-methylmorpholine complex, borane tert-butylamine complex, borane triphenylphosphine complex, borane N,N-diethylaniline complex, borane di(tert-butyl)phosphine complex, borane diphenylphosphine complex, borane ethylenediamine complex, or lithium ammonia borane. Alternative reducing strategies include the reduction of carboxylic acids via the formation of hydroxybenzotriazole esters, carboxy methyleniminium chlorides, carbonates, O-acylisoureas, acyl fluorides, cyanurates, mixed anhydrides, arylboronic anhydrides, acyl imidazolide, acyl azides, or N-acyl benzotriazoles, followed by reaction with sodium borohydride to give the corresponding alcohols.

In one embodiment, 5-hmC sequencing can use BGT and UDP-Glc to generate AbaSI-active 5-β-gmC sites, and T4-α-glucosyltransferase (AGT) and UDP-Glc to generate AbaSI-inactive 5-α-gmC sites as a negative control. In another embodiment, 5-hmC sequencing can use BGT and UDP-Glc to generate the AbaSI-active 5-β-gmC sites, and BGT and UDP-2-GlcN to generate AbaSI-inactive 5-β-2-gNmC as a negative control for 5-hmC.

In one embodiment, newly generated 5-hmC sites can be differentiated from pre-existing 5-hmC sites by sequentially transferring distinct sugar moieties from UDP-2-GlcN or native UDP-Glc using BGT.

In a further embodiment of the invention, UDP-Glc modified by any of keto, thiol, chloro, fluoro, bromo, iodo, nitro, boron, and other substituents may be transferred onto 5-hmC using BGT and may block AbaSI activity. Keto, thiol, chloro, fluoro, bromo, iodo, nitro, boron, and other substituents modifying glucose containing 5-hmC residues in a nucleic acid may facilitate cytosine modification mapping and inhibit AbaSI cleavage.

All references cited herein are incorporated by reference.

EXAMPLES

Example 1

Mapping of 5-hmC in a Nucleic Acid Sequence

The locations of 5-hmC in a nucleic acid sample can be determined using differential cleavage.

A BGT can transfer glucose ("Glc") from UDP-Glc to 5-hmC to form the glucoylated residue 5-β-2-gmC. Glucosylation enhances the sensitivity of the nucleic acid to a glucosylation-sensitive restriction enzyme such as AbaSI (see FIG. **2**). Accordingly, the identification of 5-hmC sites in a sample can be facilitating by glucosylating the nucleic acid, followed by identifying the locations of AbaSI cleavage sites.

Example 2

Exclusive 5-mC Methylome Mapping

An exemplary process for mapping 5-mC residues in a nucleic acid is depicted in FIG. **4**.

As shown in FIG. **4**, genomic DNA is treated with a BGT and UDP-2-glucosamine, converting 5-hmC residues to 5-β-2-gNmC. The resulting DNA is treated with a methylpyrimidine oxygenase from *Neisseria* (mYOX1), TET or a chemical oxidizing agent, BGT and UDP-Glc, converting existing 5-mC residues to 5-β-gmC. The DNA is digested with a 5-β-gmC-dependent restriction enzyme, such as AbaSI, cleaving at a fixed distance from 5-β-gmC and leaving a sticky end (2-base 3'-overhang). Since AbaSI does not recognize C or 5-β-2-gNmC, the only sticky ends created are those resulting from 5-β-gmC residues, which in turn are exclusively associated to 5-mC sites. A biotinylated adaptor A is then ligated onto the cleaved ends. The ligated DNA is then subjected to random fragmentation to an average size of about 200 bp. Avidin beads are used to pull out the fragments with the ligated adaptor A. After polishing the ends, adaptor P is then ligated onto the DNA fragments on the beads. The adaptor-specific PCR and the adapter ligated DNA enters the library preparation pipeline for specific sequencing platform where the end-sequencing is done from the adaptor A.

Bioinformatic analysis of the sequencing reads is facilitated by the presence of adapter A which marks the enzyme cleavage sites. After mapping the read back to the reference genome, the modified cytosine can be mapped at fixed distance away from the cleavage sites.

Example 3

Exclusive 5-fC and/or 5-caC Mapping

Exemplary processes for identifying 5-fC or 5-caC residues in a nucleic acid are depicted in FIGS. **5**A and **5**B.

Example 3A

Glucosamination

As shown in FIG. **5**A, a BGT can be used to catalyze the addition of 2-glucosamine from UDP-2-GlcN to a 5-hmC

residue, converting those residues to 5-β-2-gNmC. A reducing agent such as $NaBH_4$ (optionally in the presence of additives), can be used to reduce 5-fC and/or 5-caC to 5-hmC. The newly formed 5-hmC when combined with BGT and UDP-Glc can be subsequently converted to 5-β-gmC.

As described in Example 2, the DNA is digested with a 5-β-gmC-dependent restriction enzyme, such as AbaSI, cleaving at a fixed distance from 5-β-gmC and leaving a sticky end (2-base 3'-overhang). Since AbaSI does not recognize C or 5-β-2-gNmC, the only sticky ends created are those resulting from 5-β-gmC residues, which in turn are exclusively associated to 5-fC and/or 5-caC sites. A biotinylated adaptor A is then ligated onto the cleaved ends. The ligated DNA is then subjected to random fragmentation to an average size of about 200 bp. Avidin beads are used to pull out the fragments with the ligated adaptor A. After polishing the ends, adaptor P is then ligated onto the DNA fragments on the beads. The adaptor-specific PCR and the adapter ligated DNA enters the library preparation pipeline for specific sequencing platform where the end-sequencing is done from the adaptor A.

Bioinformatic analysis of the sequencing reads is facilitated by the presence of adapter A which marks the enzyme cleavage sites. After mapping the read back to the reference genome, the modified cytosine can be mapped at fixed distance away from the cleavage sites.

Example 3B

6-Azido-Glucose

Another exemplary process for mapping the locations of 5-fC and/or 5-caC is shown in FIG. **5**B. The process depicted in FIG. **5**B, like the process depicted in FIG. **5**A, begins with the addition of 2-glucosamine from UDP-2-GlcN to a 5-hmC residue, converting those residues to 5-β-2-gNmC in a reaction catalyzed by a BGT, and the subsequent reduction of 5-fC and/or 5-caC residues to newly-generated 5-hmC residues. In the method depicted in FIG. **5**B, UDP-6-azido-glucose (UDP-6-N3-Glc) is added to those newly-generated 5-hmC residues by a BGT. The DNA is digested with a 5-β-gmC-dependent restriction enzyme, such as AbaSI, cleaving at a fixed distance from 5-β-gmC and leaving a sticky end (2-base 3'-overhang). Since AbaSI does not recognize C or 5-β-2-gNmC, the only sticky ends created are those resulting from the azidoglucosylated residues, which in turn are exclusively associated to 5-fC and/or 5-caC sites.

The sticky ends are ligated to an adapter A. The resulting DNA is fragmented and ligated to a second adapter P1. The azido moiety can derivatized with a biotin label via azide-alkyne Huisgen cycloaddition using a copper(I) catalyst ("click chemistry") or via copper-free variants (for example, using strained cyclooctyne derivatives) and avidin beads can then be used to selectively purify the fragments containing the azidoglucosylated residues.

Example 4

Exclusive 5-caC Mapping

An exemplary process for mapping the locations of 5-caC in a nucleic acid sample is provided in FIG. **6**.

As shown in FIG. **6**, genomic DNA is treated with BGT and UDP-2-GlcN, so that substantially all 5-hmC residues are converted to 5-β-2-gNmC. The resulting DNA is treated with a reducing agent (e.g., NaBH4, converts 5-fC to 5-hmC), BGT and UDP-2-GlcN, so that all 5-fC residues are converted to 5-β-2-gNmC. Then, the resulting DNA is treated with a second reducing agent (a different reducing agent or the same reducing agent but in the presence of certain additives converts 5-caC to 5-hmC), BGT, and UDP-Glc to generate 5-β-gmC. The DNA is digested with a restriction endonuclease such as AbaSI, which cleaves 5-β-gmC exclusively associated to 5-caC sites but not 5-β-2-gNmC, 5-mC or C to leave a sticky end (2-base 3'-overhang). These sites are then identified through ligation to a biotinylated adapter, fragmentation and purification using avidin-associated beads, ligation and analysis as described in Example 2.

SEQUENCE LISTING

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<211> LENGTH: 351
<212> TYPE: PRT
<213> ORGANISM: Bacteriophage T4

<400> SEQUENCE: 1

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            20                  25                  30

Met Gly Leu Asn Val Asp Ile Ile Ser Leu Lys Asn Gly Val Tyr Thr
        35                  40                  45

Lys Ser Phe Asp Glu Val Asp Val Asn Asp Tyr Asp Arg Leu Ile Val
    50                  55                  60

Val Asn Ser Ser Ile Asn Phe Phe Gly Gly Lys Pro Asn Leu Ala Ile
65                  70                  75                  80

Leu Ser Ala Gln Lys Phe Met Ala Lys Tyr Lys Ser Lys Ile Tyr Tyr
                85                  90                  95

Leu Phe Thr Asp Ile Arg Leu Pro Phe Ser Gln Ser Trp Pro Asn Val
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-continued

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            100                 105                 110

Lys Asn Arg Pro Trp Ala Tyr Leu Tyr Thr Glu Glu Glu Leu Leu Ile
        115                 120                 125

Lys Ser Pro Ile Lys Val Ile Ser Gln Gly Ile Asn Leu Asp Ile Ala
    130                 135                 140

Lys Ala Ala His Lys Lys Val Asp Asn Val Ile Glu Phe Glu Tyr Phe
145                 150                 155                 160

Pro Ile Glu Gln Tyr Lys Ile His Met Asn Asp Phe Gln Leu Ser Lys
                165                 170                 175

Pro Thr Lys Lys Thr Leu Asp Val Ile Tyr Gly Gly Ser Phe Arg Ser
            180                 185                 190

Gly Gln Arg Glu Ser Lys Met Val Glu Phe Leu Phe Asp Thr Gly Leu
        195                 200                 205

Asn Ile Glu Phe Phe Gly Asn Ala Arg Glu Lys Gln Phe Lys Asn Pro
    210                 215                 220

Lys Tyr Pro Trp Thr Lys Ala Pro Val Phe Thr Gly Lys Ile Pro Met
225                 230                 235                 240

Asn Met Val Ser Glu Lys Asn Ser Gln Ala Ile Ala Ala Leu Ile Ile
                245                 250                 255

Gly Asp Lys Asn Tyr Asn Asp Asn Phe Ile Thr Leu Arg Val Trp Glu
            260                 265                 270

Thr Met Ala Ser Asp Ala Val Met Leu Ile Asp Glu Glu Phe Asp Thr
        275                 280                 285

Lys His Arg Ile Ile Asn Asp Ala Arg Phe Tyr Val Asn Asn Arg Ala
    290                 295                 300

Glu Leu Ile Asp Arg Val Asn Glu Leu Lys His Ser Asp Val Leu Arg
305                 310                 315                 320

Lys Glu Met Leu Ser Ile Gln His Asp Ile Leu Asn Lys Thr Arg Ala
                325                 330                 335

Lys Lys Ala Glu Trp Gln Asp Ala Phe Lys Lys Ala Ile Asp Leu
            340                 345                 350


<210> SEQ ID NO 2
<211> LENGTH: 400
<212> TYPE: PRT
<213> ORGANISM: Bacteriophage T4

<400> SEQUENCE: 2

Met Arg Ile Cys Ile Phe Met Ala Arg Gly Leu Glu Gly Cys Gly Val
1               5                   10                  15

Thr Lys Phe Ser Leu Glu Gln Arg Asp Trp Phe Ile Lys Asn Gly His
            20                  25                  30

Glu Val Thr Leu Val Tyr Ala Lys Asp Lys Ser Phe Thr Arg Thr Ser
        35                  40                  45

Ser His Asp His Lys Ser Phe Ser Ile Pro Val Ile Leu Ala Lys Glu
    50                  55                  60

Tyr Asp Lys Ala Leu Lys Leu Val Asn Asp Cys Asp Ile Leu Ile Ile
65                  70                  75                  80

Asn Ser Val Pro Ala Thr Ser Val Gln Glu Ala Thr Ile Asn Asn Tyr
                85                  90                  95

Lys Lys Leu Leu Asp Asn Ile Lys Pro Ser Ile Arg Val Val Val Tyr
            100                 105                 110

Gln His Asp His Ser Val Leu Ser Leu Arg Arg Asn Leu Gly Leu Glu
        115                 120                 125
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Glu Thr Val Arg Arg Ala Asp Val Ile Phe Ser His Ser Asp Asn Gly
    130                 135                 140

Asp Phe Asn Lys Val Leu Met Lys Glu Trp Tyr Pro Glu Thr Val Ser
145                 150                 155                 160

Leu Phe Asp Asp Ile Glu Glu Ala Pro Thr Val Tyr Asn Phe Gln Pro
                165                 170                 175

Pro Met Asp Ile Val Lys Val Arg Ser Thr Tyr Trp Lys Asp Val Ser
            180                 185                 190

Glu Ile Asn Met Asn Ile Asn Arg Trp Ile Gly Arg Thr Thr Thr Trp
        195                 200                 205

Lys Gly Phe Tyr Gln Met Phe Asp Phe His Glu Lys Phe Leu Lys Pro
    210                 215                 220

Ala Gly Lys Ser Thr Val Met Glu Gly Leu Glu Arg Ser Pro Ala Phe
225                 230                 235                 240

Ile Ala Ile Lys Glu Lys Gly Ile Pro Tyr Glu Tyr Tyr Gly Asn Arg
                245                 250                 255

Glu Ile Asp Lys Met Asn Leu Ala Pro Asn Gln Pro Ala Gln Ile Leu
            260                 265                 270

Asp Cys Tyr Ile Asn Ser Glu Met Leu Glu Arg Met Ser Lys Ser Gly
        275                 280                 285

Phe Gly Tyr Gln Leu Ser Lys Leu Asn Gln Lys Tyr Leu Gln Arg Ser
    290                 295                 300

Leu Glu Tyr Thr His Leu Glu Leu Gly Ala Cys Gly Thr Ile Pro Val
305                 310                 315                 320

Phe Trp Lys Ser Thr Gly Glu Asn Leu Lys Phe Arg Val Asp Asn Thr
                325                 330                 335

Pro Leu Thr Ser His Asp Ser Gly Ile Ile Trp Phe Asp Glu Asn Asp
            340                 345                 350

Met Glu Ser Thr Phe Glu Arg Ile Lys Glu Leu Ser Ser Asp Arg Ala
        355                 360                 365

Leu Tyr Asp Arg Glu Arg Glu Lys Ala Tyr Glu Phe Leu Tyr Gln His
    370                 375                 380

Gln Asp Ser Ser Phe Cys Phe Lys Glu Gln Phe Asp Ile Ile Thr Lys
385                 390                 395                 400


<210> SEQ ID NO 3
<211> LENGTH: 321
<212> TYPE: PRT
<213> ORGANISM: Acinetobacter baumannii

<400> SEQUENCE: 3

Leu Cys Asn Lys Ala Ser Ser Asp Leu Thr Asp Tyr Val Ile Arg Gln
1               5                   10                  15

Leu Gly Arg Thr Lys Asn Lys Arg Tyr Glu Ala Tyr Val Val Ser Arg
            20                  25                  30

Ile Ile His Leu Leu Asn Asp Phe Thr Leu Lys Phe Val Thr Gln Gln
        35                  40                  45

Phe Val Arg Leu Ser Asn Lys Lys Ile Ala Leu Thr Asp Leu Tyr Phe
    50                  55                  60

Pro Gln Leu Gly Ile His Ile Glu Val Asp Glu Gly His His Phe Leu
65                  70                  75                  80

Arg Asn Ser Lys Met Glu Tyr Ser Leu Asn Gln Ile Asp Glu Pro Leu
                85                  90                  95

Tyr Ser Ile Ser Gln Thr Glu Ser Asp Ala Met Arg Glu Glu Asp Ile
            100                 105                 110
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Ile Ser Ile Thr Gly His Lys Ile Phe Arg Val Asn Val Phe Lys Asn
        115                 120                 125

Gln Glu Gly Gln Pro Gln Asn Leu Glu Asn Ile His Gln Gln Ile Asp
    130                 135                 140

Lys Ile Ile Glu Glu Ile Lys Thr Ala Lys Asn Lys Leu Ile Glu Ala
145                 150                 155                 160

Ser Thr Phe Lys Glu Trp Asn Ile Glu Thr Glu Tyr Asn Pro Gln Thr
                165                 170                 175

Tyr Ile Asp Leu Gly Arg Ile Ser Leu Ala Asp Asn Val Val Leu Lys
            180                 185                 190

Thr Thr Lys Asp Val Cys Asn Cys Phe Gly Tyr Ser Tyr Lys Asn Tyr
        195                 200                 205

Gln Arg Gly Gly Ala Leu His Pro Tyr Lys Lys Asp Thr Leu Ile Trp
    210                 215                 220

Phe Pro Arg Leu Tyr Glu Asn Lys Asp Trp Ile Asn Thr Ile Ser Pro
225                 230                 235                 240

Asp Gly Leu Thr Ile Thr Glu Lys Ser Thr Asp Glu Thr Ile Thr Leu
                245                 250                 255

Lys Lys Leu Glu Glu Trp Lys Asn Gly Pro Gln Lys Arg Ile Val Phe
            260                 265                 270

Ala Arg Val Lys Asp Asn Leu Ser Ser Arg Ala Met Tyr Arg Phe Met
        275                 280                 285

Gly Leu Tyr Glu Phe Gln Lys Ala Asp Leu Lys Asp Gly Ala Val Trp
    290                 295                 300

Lys Arg Val Lys Cys Glu Val Gln Thr Tyr Ser Pro Lys Glu Thr Lys
305                 310                 315                 320

Cys
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<210> SEQ ID NO 4
<211> LENGTH: 318
<212> TYPE: PRT
<213> ORGANISM: Acinetobacter baumannii

<400> SEQUENCE: 4

Met Phe Ser Ser Asp Leu Thr Asp Tyr Val Ile Arg Gln Leu Gly Arg
1               5                   10                  15

Thr Lys Asn Lys Arg Tyr Glu Ala Tyr Val Val Ser Arg Ile Ile His
            20                  25                  30

Leu Leu Asn Asp Phe Thr Leu Lys Phe Val Thr Gln Gln Phe Val Arg
        35                  40                  45

Leu Ser Asn Lys Lys Ile Ala Leu Thr Asp Leu Tyr Phe Pro Gln Leu
    50                  55                  60

Gly Ile His Ile Glu Val Asp Glu Glu His His Phe Leu Arg Asn Ser
65                  70                  75                  80

Lys Met Glu Tyr Ser Leu Asn Gln Ile Asp Glu Pro Leu Tyr Ser Ile
                85                  90                  95

Ser His Thr Glu Ser Asp Ala Met Arg Glu Glu Asp Ile Ile Ser Ile
            100                 105                 110

Thr Gly His Lys Ile Phe Arg Val Asn Val Phe Lys Asn Gln Glu Gly
        115                 120                 125

Gln Pro Gln Asn Leu Glu Ser Ile His Gln Gln Ile Asp Lys Ile Ile
    130                 135                 140

Glu Lys Ile Lys Thr Ala Lys Asn Lys Leu Ile Glu Ala Ser Thr Phe
145                 150                 155                 160
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Lys Glu Trp Asn Ile Glu Thr Glu Tyr Asn Pro Gln Thr Tyr Ile Asp
165 170 175

Leu Gly Arg Ile Ser Leu Ala Asp Asn Val Val Leu Lys Thr Thr Lys
180 185 190

Asp Val Cys Asn Cys Phe Gly Tyr Asn Tyr Lys Asn Tyr Gln Arg Gly
195 200 205

Gly Ala Leu His Pro Tyr Glu Lys Asp Thr Leu Ile Trp Phe Pro Arg
210 215 220

Leu Tyr Glu Asn Lys Asp Trp Phe Asn Thr Ile Ser Pro Asp Gly Leu
225 230 235 240

Thr Ile Thr Glu Lys Ser Thr Asp Glu Ala Ile Thr Leu Lys Lys Leu
245 250 255

Glu Glu Trp Lys Asn Gly Pro Gln Lys Arg Ile Val Phe Ala Arg Val
260 265 270

Lys Asp Asn Leu Ser Ser Arg Ala Met Tyr Arg Phe Met Gly Leu Tyr
275 280 285

Glu Phe Gln Lys Ala Asp Leu Lys Asp Gly Ala Val Trp Lys Arg Val
290 295 300

Glu Cys Glu Val Gln Thr Tyr Ser Pro Lys Glu Thr Lys Cys
305 310 315

<210> SEQ ID NO 5
<211> LENGTH: 318
<212> TYPE: PRT
<213> ORGANISM: Acinetobacter baumannii

<400> SEQUENCE: 5

Met Phe Ser Ser Asp Leu Thr Asp Tyr Val Ile Arg Gln Leu Gly Arg
1 5 10 15

Thr Lys Asn Lys Arg Tyr Glu Ala Tyr Val Val Ser Arg Ile Ile His
20 25 30

Leu Leu Asn Asp Phe Thr Leu Lys Phe Val Thr Gln Gln Phe Val Arg
35 40 45

Leu Ser Asn Lys Lys Ile Ala Leu Thr Asp Leu Tyr Phe Pro Gln Leu
50 55 60

Gly Ile His Ile Glu Val Asp Glu Gly His His Phe Leu Arg Asn Ser
65 70 75 80

Lys Met Glu Tyr Ser Leu Asn Gln Ile Asp Glu Pro Leu Tyr Ser Ile
85 90 95

Ser Gln Thr Glu Ser Asp Ala Met Arg Glu Glu Asp Ile Ile Ser Ile
100 105 110

Thr Gly His Lys Ile Phe Arg Val Asn Val Phe Lys Asn Gln Glu Gly
115 120 125

Gln Pro Gln Asn Leu Glu Ser Ile His Gln Gln Ile Asp Lys Ile Ile
130 135 140

Glu Glu Ile Lys Thr Ala Lys Asn Lys Leu Ile Glu Ala Ser Thr Phe
145 150 155 160

Lys Glu Trp Asn Ile Glu Thr Glu Tyr Asn Pro Gln Thr Tyr Ile Asp
165 170 175

Leu Gly Arg Ile Ser Leu Ala Asp Asn Val Val Leu Lys Thr Thr Lys
180 185 190

Asp Val Cys Asn Cys Phe Gly Tyr Asn Tyr Lys Asn Tyr Gln Arg Gly
195 200 205

Gly Ala Leu His Pro Tyr Glu Lys Asp Thr Leu Ile Trp Phe Pro Arg

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    210                 215                 220

Leu Tyr Glu Asn Lys Asp Trp Ile Asn Thr Ile Ser Pro Asp Gly Leu
225                 230                 235                 240

Thr Ile Thr Glu Lys Ser Thr Asp Glu Thr Ile Thr Leu Lys Lys Leu
                245                 250                 255

Glu Glu Trp Lys Asn Gly Pro Gln Lys Arg Ile Val Phe Ala Arg Val
            260                 265                 270

Lys Asp Asn Leu Ser Ser Arg Ala Met Tyr Arg Phe Met Gly Leu Tyr
        275                 280                 285

Glu Phe Gln Lys Ala Asp Leu Lys Asp Gly Ala Val Trp Lys Arg Val
    290                 295                 300

Lys Cys Glu Val Gln Thr Tyr Ser Pro Lys Glu Thr Lys Cys
305                 310                 315
```

<210> SEQ ID NO 6
<211> LENGTH: 318
<212> TYPE: PRT
<213> ORGANISM: Acinetobacter baumannii

<400> SEQUENCE: 6

```
Met Phe Ser Ser Asp Leu Thr Asp Tyr Val Ile Arg Gln Leu Gly Arg
1               5                   10                  15

Thr Lys Asn Lys Arg Tyr Glu Thr Tyr Val Val Ser Arg Ile Ile His
            20                  25                  30

Leu Leu Asn Asp Phe Thr Leu Lys Phe Val Thr Gln Gln Phe Val Arg
        35                  40                  45

Leu Ser Asn Lys Lys Ile Ala Leu Thr Asp Leu Tyr Phe Pro Gln Leu
    50                  55                  60

Gly Ile His Ile Glu Val Asp Glu Glu His His Phe Leu Arg Asn Ser
65                  70                  75                  80

Lys Met Glu Tyr Ser Leu Asn Gln Ile Asp Glu Pro Leu Tyr Ser Ile
                85                  90                  95

Ser His Thr Glu Ser Asp Ala Met Arg Glu Glu Asp Ile Ile Ser Ile
            100                 105                 110

Thr Gly His Lys Ile Phe Arg Val Asn Val Phe Lys Asn Gln Glu Gly
        115                 120                 125

Gln Pro Gln Asn Leu Glu Ser Ile His Gln Gln Ile Asp Lys Ile Ile
    130                 135                 140

Glu Glu Ile Lys Thr Ala Lys Asn Lys Leu Ile Glu Ala Ser Thr Phe
145                 150                 155                 160

Lys Glu Trp Asn Ile Glu Thr Glu Tyr Asn Pro Gln Thr Tyr Ile Asp
                165                 170                 175

Leu Gly Arg Ile Ser Leu Ala Asp Asn Val Val Leu Lys Thr Thr Lys
            180                 185                 190

Asp Val Cys Asn Cys Phe Gly Tyr Asn Tyr Lys Asn Tyr Gln Arg Gly
        195                 200                 205

Gly Ala Leu His Pro Tyr Lys Lys Asp Thr Leu Ile Trp Phe Pro Arg
    210                 215                 220

Leu Tyr Glu Asn Lys Asp Trp Ile Asn Thr Ile Ser Pro Asp Gly Leu
225                 230                 235                 240

Thr Ile Thr Glu Lys Ser Thr Asp Glu Thr Ile Thr Leu Lys Lys Leu
                245                 250                 255

Glu Glu Trp Lys Asn Gly Pro Gln Lys Arg Ile Val Phe Ala Arg Val
            260                 265                 270
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Lys Asp Asn Leu Ser Ser Arg Ala Met Tyr Arg Phe Met Gly Leu Tyr
275 280 285

Glu Phe Gln Lys Ala Asp Leu Lys Asp Gly Ala Val Trp Lys Arg Val
290 295 300

Glu Cys Glu Val Gln Thr Tyr Ser Pro Lys Glu Thr Lys Cys
305 310 315

<210> SEQ ID NO 7
<211> LENGTH: 268
<212> TYPE: PRT
<213> ORGANISM: Acinetobacter baumannii

<400> SEQUENCE: 7

Ile Cys Arg Val Gln Arg Thr Asp Leu Tyr Phe Pro Gln Leu Gly Ile
1 5 10 15

His Ile Glu Val Asp Glu Gly His His Phe Leu Arg Asn Ser Lys Met
20 25 30

Glu Tyr Ser Leu Asn Gln Ile Asp Glu Pro Leu Tyr Ser Ile Ser Gln
35 40 45

Thr Glu Ser Asp Ala Met Arg Glu Glu Asp Ile Ile Ser Ile Thr Gly
50 55 60

His Lys Ile Phe Arg Val Asn Val Tyr Lys Asn Gln Glu Gly Gln Pro
65 70 75 80

Gln Asn Leu Glu Ser Ile His Gln Gln Ile Asp Lys Ile Ile Glu Glu
85 90 95

Ile Lys Thr Ala Lys Asn Lys Leu Ile Glu Ala Ser Thr Phe Lys Glu
100 105 110

Trp Asn Ile Glu Thr Glu Tyr Asn Pro Gln Thr Tyr Ile Asn Leu Gly
115 120 125

Arg Ile Ser Leu Ala Asp Asn Val Val Leu Lys Thr Thr Lys Asp Val
130 135 140

Cys Asn Cys Phe Gly Tyr Asn Tyr Lys Asn Tyr Gln Arg Gly Gly Ala
145 150 155 160

Ile His Pro Tyr Glu Glu Asp Thr Leu Ile Trp Phe Pro Arg Leu Tyr
165 170 175

Glu Asn Lys Asp Trp Ile Asn Thr Ile Ser Pro Asp Gly Leu Thr Ile
180 185 190

Thr Glu Lys Ser Thr Asp Glu Thr Ile Thr Leu Lys Lys Leu Glu Glu
195 200 205

Trp Lys Asn Gly Pro Gln Lys Arg Ile Val Phe Ala Arg Val Lys Asp
210 215 220

Asn Leu Asn Ser Arg Ala Met Tyr Arg Phe Met Gly Leu Tyr Lys Phe
225 230 235 240

Gln Lys Ala Asp Leu Lys Asp Gly Ala Val Trp Lys Arg Val Glu Cys
245 250 255

Glu Val Gln Thr Tyr Ser Pro Lys Glu Thr Lys Cys
260 265

<210> SEQ ID NO 8
<211> LENGTH: 318
<212> TYPE: PRT
<213> ORGANISM: Acinetobacter baumannii

<400> SEQUENCE: 8

Met Phe Ser Ser Asp Leu Thr Asp Tyr Val Ile Arg Gln Leu Gly Arg
1 5 10 15

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```
Thr Lys Asn Lys Arg Tyr Glu Ala Tyr Val Val Ser Arg Ile Ile His
            20                  25                  30

Leu Leu Asn Asp Ile Thr Leu Lys Phe Val Thr Gln Gln Phe Val Arg
        35                  40                  45

Leu Ser Asn Lys Lys Ile Ala Leu Thr Asp Leu Tyr Phe Pro Gln Leu
    50                  55                  60

Gly Ile His Ile Glu Val Asp Glu Gly His His Phe Leu Arg Asn Ser
65                  70                  75                  80

Lys Met Glu Tyr Ser Leu Asn Gln Ile Asp Glu Pro Leu Tyr Ser Ile
                85                  90                  95

Ser Gln Thr Glu Ser Asp Ala Met Arg Glu Glu Asp Ile Ile Ser Ile
            100                 105                 110

Thr Glu His Lys Ile Phe Arg Val Asn Val Tyr Lys Asn Gln Glu Gly
        115                 120                 125

Gln Pro Gln Asn Leu Glu Ser Ile His Gln Gln Ile Asp Lys Ile Ile
    130                 135                 140

Glu Glu Ile Lys Thr Ala Lys Asn Lys Leu Val Glu Glu Phe Lys Phe
145                 150                 155                 160

Lys Glu Trp Asn Ile Glu Thr Glu Tyr Asn Pro Gln Thr Tyr Ile Asp
                165                 170                 175

Leu Gly Arg Ile Ser Leu Ala Asp Asn Val Val Leu Lys Thr Thr Lys
            180                 185                 190

Asp Val Cys Asn Cys Phe Gly Tyr Asn Tyr Lys Asn Tyr Gln Arg Gly
        195                 200                 205

Gly Ala Leu His Pro Tyr Glu Lys Asp Thr Leu Ile Trp Phe Pro Arg
    210                 215                 220

Leu Tyr Glu Asn Lys Asp Trp Ile Asn Thr Ile Ser Pro Asp Gly Leu
225                 230                 235                 240

Thr Ile Thr Glu Lys Ser Thr Asp Glu Thr Ile Thr Leu Lys Lys Leu
                245                 250                 255

Glu Glu Trp Lys Asn Gly Pro Gln Lys Arg Ile Val Phe Ala Arg Val
            260                 265                 270

Lys Asp Asn Leu Ser Ser Arg Ala Met Tyr Arg Phe Met Gly Leu Tyr
        275                 280                 285

Glu Phe Gln Lys Ala Asp Leu Lys Asp Gly Ala Val Trp Lys Arg Val
    290                 295                 300

Lys Cys Glu Val Gln Thr Tyr Ser Pro Lys Glu Thr Lys Cys
305                 310                 315


<210> SEQ ID NO 9
<211> LENGTH: 318
<212> TYPE: PRT
<213> ORGANISM: Acinetobacter calcoaceticus

<400> SEQUENCE: 9

Met Phe Ser Ser Asp Leu Thr Asp Tyr Val Ile Arg Gln Leu Gly Arg
1               5                   10                  15

Thr Lys Asn Lys Arg Tyr Glu Ala Tyr Val Val Ser Arg Ile Ile His
            20                  25                  30

Leu Leu Asn Asp Phe Thr Leu Lys Phe Val Thr Gln Gln Phe Val Arg
        35                  40                  45

Leu Ser Asn Lys Lys Ile Ala Leu Thr Asp Leu Tyr Phe Pro Gln Leu
    50                  55                  60

Asp Ile His Ile Glu Val Asp Glu Gly His His Phe Leu Arg Asn Ser
65                  70                  75                  80
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```
Lys Met Glu Tyr Ser Leu Asn Gln Ile Asp Glu Pro Leu Tyr Ser Ile
                85                  90                  95

Ser Gln Thr Glu Ser Asp Ala Met Arg Glu Glu Asp Ile Ile Ser Ile
            100                 105                 110

Thr Gly His Lys Ile Phe Arg Val Asn Val Tyr Lys Asn Gln Glu Gly
        115                 120                 125

Glu Pro Gln Asn Leu Glu Ser Ile His Gln Gln Ile Asp Lys Ile Ile
    130                 135                 140

Glu Glu Ile Lys Val Ala Lys Asn Lys Gln Ile Lys Ala Ser Thr Phe
145                 150                 155                 160

Lys Glu Trp Asn Ile Glu Thr Glu Tyr Asn Pro Gln Thr Tyr Ile Asp
                165                 170                 175

Leu Gly Ser Ile Ser Leu Ala Asp Asn Val Val Leu Lys Thr Thr Lys
            180                 185                 190

Asp Val Cys Asn Cys Phe Gly Tyr Asn Tyr Lys Asn Tyr Gln Arg Gly
        195                 200                 205

Gly Ala Ile His Pro Tyr Glu Lys Asp Thr Leu Ile Trp Phe Pro Arg
    210                 215                 220

Leu Tyr Glu Asn Lys Asp Trp Ile Asn Thr Ile Ser Pro Asp Gly Leu
225                 230                 235                 240

Thr Ile Thr Glu Lys Ser Thr Asp Glu Ala Ile Thr Leu Lys Lys Leu
                245                 250                 255

Glu Glu Trp Lys Asn Gly Pro Gln Lys Arg Ile Val Phe Ala Arg Val
            260                 265                 270

Lys Asp Asn Leu Ser Ser Arg Ala Met Tyr Arg Phe Met Gly Leu Tyr
        275                 280                 285

Glu Phe Gln Lys Ala Asp Leu Lys Asp Gly Ala Val Trp Lys Arg Glu
    290                 295                 300

Gly Cys Lys Val Gln Thr Tyr Ser Pro Lys Glu Ala Lys Cys
305                 310                 315
```

<210> SEQ ID NO 10
<211> LENGTH: 296
<212> TYPE: PRT
<213> ORGANISM: Paraprevotella xylaniphila

<400> SEQUENCE: 10

```
Val Lys Tyr Gly Thr Asn Ser Lys Phe Lys Asp Met Asp Tyr Lys Leu
1               5                   10                  15

Asp Tyr Met Glu Arg Leu Phe Ala Lys Ile Ser Lys Lys Lys Thr Glu
            20                  25                  30

Ser Tyr Val Ile Ser Arg Ile Trp His Gln Leu Asp Asp Asp Arg Val
        35                  40                  45

Lys Phe Val Val Gln Gln Tyr Ile Arg Arg Thr Gln Asp Lys Tyr Ala
    50                  55                  60

Leu Ala Asp Leu Tyr Leu Pro Gln Leu Asn Ile Phe Ile Glu Ile Asn
65                  70                  75                  80

Glu Pro Phe His Lys Asn Asn Thr Glu Ile Asp Lys Ile Arg Asn Glu
                85                  90                  95

Glu Ile Leu Asn Ile Thr Asn Ser Lys Pro Ile Ile Ile Asp Cys Asp
            100                 105                 110

Asn Asn Ile Gln Glu Ile His His Gln Ile Thr Asp Val Val Ser Leu
        115                 120                 125

Ile Lys Gln Cys Ile Gln Glu Met Gly Asp Asn Phe Gln Pro Trp Asp
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    130                 135                 140

Asp Val Ser Thr Leu Ser Val Glu Tyr His Arg Asn Lys Gly Tyr Leu
145                 150                 155                 160

Lys Val Asp Asp Asn Glu Cys Leu Arg Thr Thr Asp Asp Val Ala Glu
                165                 170                 175

Thr Phe Gly Thr Lys Pro Lys His Arg Gly Phe Leu Arg Ala Ser Gly
            180                 185                 190

Ala Ala Val Pro Asn Lys Lys Asn Glu Ile Ile Trp Trp Pro Asn Thr
        195                 200                 205

Glu His Arg Leu Trp Cys Asn Glu Leu Ser Glu Asp Gly Met Phe Ile
    210                 215                 220

Tyr Glu Tyr Pro Lys Ala Glu Asn Lys Arg Thr Ala His Leu Lys Gln
225                 230                 235                 240

Trp Leu Ser Ala Pro Glu Glu Thr Arg Ile Thr Phe Leu Arg Tyr Lys
                245                 250                 255

Asp Asp Leu Gly Phe Cys Phe Tyr Arg Phe Val Gly Val Phe Asn Leu
            260                 265                 270

Asn Lys Glu Lys Ser Ile Lys Glu Asn Lys Cys Val Trp Glu Arg Val
        275                 280                 285

Ser Asn Thr Tyr Gln Leu Asn Val
    290                 295


<210> SEQ ID NO 11
<211> LENGTH: 321
<212> TYPE: PRT
<213> ORGANISM: Naegleria gruberi

<400> SEQUENCE: 11

Met Thr Thr Phe Lys Gln Gln Thr Ile Lys Glu Lys Glu Thr Lys Arg
1               5                   10                  15

Lys Tyr Cys Ile Lys Gly Thr Thr Ala Asn Leu Thr Gln Thr His Pro
            20                  25                  30

Asn Gly Pro Val Cys Val Asn Arg Gly Glu Glu Val Ala Asn Thr Thr
        35                  40                  45

Thr Leu Leu Asp Ser Gly Gly Gly Ile Asn Lys Lys Ser Leu Leu Gln
    50                  55                  60

Asn Leu Leu Ser Lys Cys Lys Thr Thr Phe Gln Gln Ser Phe Thr Asn
65                  70                  75                  80

Ala Asn Ile Thr Leu Lys Asp Glu Lys Trp Leu Lys Asn Val Arg Thr
                85                  90                  95

Ala Tyr Phe Val Cys Asp His Asp Gly Ser Val Glu Leu Ala Tyr Leu
            100                 105                 110

Pro Asn Val Leu Pro Lys Glu Leu Val Glu Glu Phe Thr Glu Lys Phe
        115                 120                 125

Glu Ser Ile Gln Thr Gly Arg Lys Lys Asp Thr Gly Tyr Ser Gly Ile
    130                 135                 140

Leu Asp Asn Ser Met Pro Phe Asn Tyr Val Thr Ala Asp Leu Ser Gln
145                 150                 155                 160

Glu Leu Gly Gln Tyr Leu Ser Glu Ile Val Asn Pro Gln Ile Asn Tyr
                165                 170                 175

Tyr Ile Ser Lys Leu Leu Thr Cys Val Ser Ser Arg Thr Ile Asn Tyr
            180                 185                 190

Leu Val Ser Leu Asn Asp Ser Tyr Tyr Ala Leu Asn Asn Cys Leu Tyr
        195                 200                 205
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```
Pro Ser Thr Ala Phe Asn Ser Leu Lys Pro Ser Asn Asp Gly His Arg
    210                 215                 220

Ile Arg Lys Pro His Lys Asp Asn Leu Asp Ile Thr Pro Ser Ser Leu
225                 230                 235                 240

Phe Tyr Phe Gly Asn Phe Gln Asn Thr Glu Gly Tyr Leu Glu Leu Thr
                245                 250                 255

Asp Lys Asn Cys Lys Val Phe Val Gln Pro Gly Asp Val Leu Phe Phe
            260                 265                 270

Lys Gly Asn Glu Tyr Lys His Val Val Ala Asn Ile Thr Ser Gly Trp
        275                 280                 285

Arg Ile Gly Leu Val Tyr Phe Ala His Lys Gly Ser Lys Thr Lys Pro
    290                 295                 300

Tyr Tyr Glu Asp Thr Gln Lys Asn Ser Leu Lys Ile His Lys Glu Thr
305                 310                 315                 320

Lys
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<210> SEQ ID NO 12
<211> LENGTH: 2039
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 12

```
Met Ser Arg Ser Arg Pro Ala Lys Pro Ser Lys Ser Val Lys Thr Lys
1               5                   10                  15

Leu Gln Lys Lys Lys Asp Ile Gln Met Lys Thr Lys Thr Ser Lys Gln
            20                  25                  30

Ala Val Arg His Gly Ala Ser Ala Lys Ala Val Asn Pro Gly Lys Pro
        35                  40                  45

Lys Gln Leu Ile Lys Arg Arg Asp Gly Lys Lys Glu Thr Glu Asp Lys
    50                  55                  60

Thr Pro Thr Pro Ala Pro Ser Phe Leu Thr Arg Ala Gly Ala Ala Arg
65                  70                  75                  80

Met Asn Arg Asp Arg Asn Gln Val Leu Phe Gln Asn Pro Asp Ser Leu
                85                  90                  95

Thr Cys Asn Gly Phe Thr Met Ala Leu Arg Arg Thr Ser Leu Ser Trp
            100                 105                 110

Arg Leu Ser Gln Arg Pro Val Val Thr Pro Lys Pro Lys Lys Val Pro
        115                 120                 125

Pro Ser Lys Lys Gln Cys Thr His Asn Ile Gln Asp Glu Pro Gly Val
    130                 135                 140

Lys His Ser Glu Asn Asp Ser Val Pro Ser Gln His Ala Thr Val Ser
145                 150                 155                 160

Pro Gly Thr Glu Asn Gly Glu Gln Asn Arg Cys Leu Val Glu Gly Glu
                165                 170                 175

Ser Gln Glu Ile Thr Gln Ser Cys Pro Val Phe Glu Glu Arg Ile Glu
            180                 185                 190

Asp Thr Gln Ser Cys Ile Ser Ala Ser Gly Asn Leu Glu Ala Glu Ile
        195                 200                 205

Ser Trp Pro Leu Glu Gly Thr His Cys Glu Glu Leu Leu Ser His Gln
    210                 215                 220

Thr Ser Asp Asn Glu Cys Thr Ser Pro Gln Glu Cys Ala Pro Leu Pro
225                 230                 235                 240

Gln Arg Ser Thr Ser Glu Val Thr Ser Gln Lys Asn Thr Ser Asn Gln
                245                 250                 255
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Leu Ala Asp Leu Ser Ser Gln Val Glu Ser Ile Lys Leu Ser Asp Pro
            260                 265                 270

Ser Pro Asn Pro Thr Gly Ser Asp His Asn Gly Phe Pro Asp Ser Ser
        275                 280                 285

Phe Arg Ile Val Pro Glu Leu Asp Leu Lys Thr Cys Met Pro Leu Asp
    290                 295                 300

Glu Ser Val Tyr Pro Thr Ala Leu Ile Arg Phe Ile Leu Ala Gly Ser
305                 310                 315                 320

Gln Pro Asp Val Phe Asp Thr Lys Pro Gln Glu Lys Thr Leu Ile Thr
                325                 330                 335

Thr Pro Glu Gln Val Gly Ser His Pro Asn Gln Val Leu Asp Ala Thr
            340                 345                 350

Ser Val Leu Gly Gln Ala Phe Ser Thr Leu Pro Leu Gln Trp Gly Phe
        355                 360                 365

Ser Gly Ala Asn Leu Val Gln Val Glu Ala Leu Gly Lys Gly Ser Asp
    370                 375                 380

Ser Pro Glu Asp Leu Gly Ala Ile Thr Met Leu Asn Gln Gln Glu Thr
385                 390                 395                 400

Val Ala Met Asp Met Asp Arg Asn Ala Thr Pro Asp Leu Pro Ile Phe
                405                 410                 415

Leu Pro Lys Pro Pro Asn Thr Val Ala Thr Tyr Ser Ser Pro Leu Leu
            420                 425                 430

Gly Pro Glu Pro His Ser Ser Thr Ser Cys Gly Leu Glu Val Gln Gly
        435                 440                 445

Ala Thr Pro Ile Leu Thr Leu Asp Ser Gly His Thr Pro Gln Leu Pro
    450                 455                 460

Pro Asn Pro Glu Ser Ser Ser Val Pro Leu Val Ile Ala Ala Asn Gly
465                 470                 475                 480

Thr Arg Ala Glu Lys Gln Phe Gly Thr Ser Leu Phe Pro Ala Val Pro
                485                 490                 495

Gln Gly Phe Thr Val Ala Ala Glu Asn Glu Val Gln His Ala Pro Leu
            500                 505                 510

Asp Leu Thr Gln Gly Ser Gln Ala Ala Pro Ser Lys Leu Glu Gly Glu
        515                 520                 525

Ile Ser Arg Val Ser Ile Thr Gly Ser Ala Asp Val Lys Ala Thr Ala
    530                 535                 540

Met Ser Met Pro Val Thr Gln Ala Ser Thr Ser Ser Pro Pro Cys Asn
545                 550                 555                 560

Ser Thr Pro Pro Met Val Glu Arg Arg Lys Arg Lys Ala Cys Gly Val
                565                 570                 575

Cys Glu Pro Cys Gln Gln Lys Ala Asn Cys Gly Glu Cys Thr Tyr Cys
            580                 585                 590

Lys Asn Arg Lys Asn Ser His Gln Ile Cys Lys Lys Arg Lys Cys Glu
        595                 600                 605

Val Leu Lys Lys Lys Pro Glu Ala Thr Ser Gln Ala Gln Val Thr Lys
    610                 615                 620

Glu Asn Lys Arg Pro Gln Arg Glu Lys Lys Pro Lys Val Leu Lys Thr
625                 630                 635                 640

Asp Phe Asn Asn Lys Pro Val Asn Gly Pro Lys Ser Glu Ser Met Asp
                645                 650                 655

Cys Ser Arg Arg Gly His Gly Glu Glu Glu Gln Arg Leu Asp Leu Ile
            660                 665                 670

Thr His Pro Leu Glu Asn Val Arg Lys Asn Ala Gly Gly Met Thr Gly
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        675                 680                 685

Ile Glu Val Glu Lys Trp Ala Pro Asn Lys Lys Ser His Leu Ala Glu
    690                 695                 700

Gly Gln Val Lys Gly Ser Cys Asp Ala Asn Leu Thr Gly Val Glu Asn
705                 710                 715                 720

Pro Gln Pro Ser Glu Asp Asp Lys Gln Gln Thr Asn Pro Ser Pro Thr
                725                 730                 735

Phe Ala Gln Thr Ile Arg Asn Gly Met Lys Asn Val His Cys Leu Pro
            740                 745                 750

Thr Asp Thr His Leu Pro Leu Asn Lys Leu Asn His Glu Glu Phe Ser
        755                 760                 765

Lys Ala Leu Gly Asn Asn Ser Ser Lys Leu Leu Thr Asp Pro Ser Asn
    770                 775                 780

Cys Lys Asp Ala Met Ser Val Thr Thr Ser Gly Gly Glu Cys Asp His
785                 790                 795                 800

Leu Lys Gly Pro Arg Asn Thr Leu Leu Phe Gln Lys Pro Gly Leu Asn
                805                 810                 815

Cys Arg Ser Gly Ala Glu Pro Thr Ile Phe Asn Asn His Pro Asn Thr
            820                 825                 830

His Ser Ala Gly Ser Arg Pro His Pro Pro Glu Lys Val Pro Asn Lys
        835                 840                 845

Glu Pro Lys Asp Gly Ser Pro Val Gln Pro Ser Leu Leu Ser Leu Met
    850                 855                 860

Lys Asp Arg Arg Leu Thr Leu Glu Gln Val Val Ala Ile Glu Ala Leu
865                 870                 875                 880

Thr Gln Leu Ser Glu Ala Pro Ser Glu Ser Ser Ser Pro Ser Lys Pro
                885                 890                 895

Glu Lys Asp Glu Glu Ala His Gln Lys Thr Ala Ser Leu Leu Asn Ser
            900                 905                 910

Cys Lys Ala Ile Leu His Ser Val Arg Lys Asp Leu Gln Asp Pro Asn
        915                 920                 925

Val Gln Gly Lys Gly Leu His His Asp Thr Val Val Phe Asn Gly Gln
    930                 935                 940

Asn Arg Thr Phe Lys Ser Pro Asp Ser Phe Ala Thr Asn Gln Ala Leu
945                 950                 955                 960

Ile Lys Ser Gln Gly Tyr Pro Ser Ser Pro Thr Ala Glu Lys Lys Gly
                965                 970                 975

Ala Ala Gly Gly Arg Ala Pro Phe Asp Gly Phe Glu Asn Ser His Pro
            980                 985                 990

Leu Pro Ile Glu Ser His Asn Leu  Glu Asn Cys Ser Gln  Val Leu Ser
        995                 1000                1005

Cys Asp  Gln Asn Leu Ser Ser  His Asp Pro Ser Cys  Gln Asp Ala
    1010                1015                1020

Pro Tyr  Ser Gln Ile Glu Glu  Asp Val Ala Ala Gln  Leu Thr Gln
    1025                1030                1035

Leu Ala  Ser Thr Ile Asn His  Ile Asn Ala Glu Val  Arg Asn Ala
    1040                1045                1050

Glu Ser  Thr Pro Glu Ser Leu  Val Ala Lys Asn Thr  Lys Gln Lys
    1055                1060                1065

His Ser  Gln Glu Lys Arg Met  Val His Gln Lys Pro  Pro Ser Ser
    1070                1075                1080

Thr Gln  Thr Lys Pro Ser Val  Pro Ser Ala Lys Pro  Lys Lys Ala
    1085                1090                1095
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Gln Lys  Lys Ala Arg Ala Thr  Pro His Ala Asn Lys  Arg Lys Lys
    1100                 1105                 1110

Lys Pro  Pro Ala Arg Ser Ser  Gln Glu Asn Asp Gln  Lys Lys Gln
    1115                 1120                 1125

Glu Gln  Leu Ala Ile Glu Tyr  Ser Lys Met His Asp  Ile Trp Met
    1130                 1135                 1140

Ser Ser  Lys Phe Gln Arg Phe  Gly Gln Ser Ser Pro  Arg Ser Phe
    1145                 1150                 1155

Pro Val  Leu Leu Arg Asn Ile  Pro Val Phe Asn Gln  Ile Leu Lys
    1160                 1165                 1170

Pro Val  Thr Gln Ser Lys Thr  Pro Ser Gln His Asn  Glu Leu Phe
    1175                 1180                 1185

Pro Pro  Ile Asn Gln Ile Lys  Phe Thr Arg Asn Pro  Glu Leu Ala
    1190                 1195                 1200

Lys Glu  Lys Val Lys Val Glu  Pro Ser Asp Ser Leu  Pro Thr Cys
    1205                 1210                 1215

Gln Phe  Lys Thr Glu Ser Gly  Gly Gln Thr Phe Ala  Glu Pro Ala
    1220                 1225                 1230

Asp Asn  Ser Gln Gly Gln Pro  Met Val Ser Val Asn  Gln Glu Ala
    1235                 1240                 1245

His Pro  Leu Pro Gln Ser Pro  Pro Ser Asn Gln Cys  Ala Asn Ile
    1250                 1255                 1260

Met Ala  Gly Ala Ala Gln Thr  Gln Phe His Leu Gly  Ala Gln Glu
    1265                 1270                 1275

Asn Leu  Val His Gln Ile Pro  Pro Pro Thr Leu Pro  Gly Thr Ser
    1280                 1285                 1290

Pro Asp  Thr Leu Leu Pro Asp  Pro Ala Ser Ile Leu  Arg Lys Gly
    1295                 1300                 1305

Lys Val  Leu His Phe Asp Gly  Ile Thr Val Val Thr  Glu Lys Arg
    1310                 1315                 1320

Glu Ala  Gln Thr Ser Ser Asn  Gly Pro Leu Gly Pro  Thr Thr Asp
    1325                 1330                 1335

Ser Ala  Gln Ser Glu Phe Lys  Glu Ser Ile Met Asp  Leu Leu Ser
    1340                 1345                 1350

Lys Pro  Ala Lys Asn Leu Ile  Ala Gly Leu Lys Glu  Gln Glu Ala
    1355                 1360                 1365

Ala Pro  Cys Asp Cys Asp Gly  Gly Thr Gln Lys Glu  Lys Gly Pro
    1370                 1375                 1380

Tyr Tyr  Thr His Leu Gly Ala  Gly Pro Ser Val Ala  Ala Val Arg
    1385                 1390                 1395

Glu Leu  Met Glu Thr Arg Phe  Gly Gln Lys Gly Lys  Ala Ile Arg
    1400                 1405                 1410

Ile Glu  Lys Ile Val Phe Thr  Gly Lys Glu Gly Lys  Ser Ser Gln
    1415                 1420                 1425

Gly Cys  Pro Val Ala Lys Trp  Val Ile Arg Arg Ser  Gly Pro Glu
    1430                 1435                 1440

Glu Lys  Leu Ile Cys Leu Val  Arg Glu Arg Val Asp  His His Cys
    1445                 1450                 1455

Ser Thr  Ala Val Ile Val Val  Leu Ile Leu Leu Trp  Glu Gly Ile
    1460                 1465                 1470

Pro Arg  Leu Met Ala Asp Arg  Leu Tyr Lys Glu Leu  Thr Glu Asn
    1475                 1480                 1485
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Leu Arg  Ser Tyr Ser Gly His  Pro Thr Asp Arg Arg  Cys Thr Leu
    1490                1495                1500

Asn Lys  Lys Arg Thr Cys Thr  Cys Gln Gly Ile Asp  Pro Lys Thr
    1505                1510                1515

Cys Gly  Ala Ser Phe Ser Phe  Gly Cys Ser Trp Ser  Met Tyr Phe
    1520                1525                1530

Asn Gly  Cys Lys Phe Gly Arg  Ser Glu Asn Pro Arg  Lys Phe Arg
    1535                1540                1545

Leu Ala  Pro Asn Tyr Pro Leu  His Asn Tyr Tyr Lys  Arg Ile Thr
    1550                1555                1560

Gly Met  Ser Ser Glu Gly Ser  Asp Val Lys Thr Gly  Trp Ile Ile
    1565                1570                1575

Pro Asp  Arg Lys Thr Leu Ile  Ser Arg Glu Glu Lys  Gln Leu Glu
    1580                1585                1590

Lys Asn  Leu Gln Glu Leu Ala  Thr Val Leu Ala Pro  Leu Tyr Lys
    1595                1600                1605

Gln Met  Ala Pro Val Ala Tyr  Gln Asn Gln Val Glu  Tyr Glu Glu
    1610                1615                1620

Val Ala  Gly Asp Cys Arg Leu  Gly Asn Glu Glu Gly  Arg Pro Phe
    1625                1630                1635

Ser Gly  Val Thr Cys Cys Met  Asp Phe Cys Ala His  Ser His Lys
    1640                1645                1650

Asp Ile  His Asn Met His Asn  Gly Ser Thr Val Val  Cys Thr Leu
    1655                1660                1665

Ile Arg  Ala Asp Gly Arg Asp  Thr Asn Cys Pro Glu  Asp Glu Gln
    1670                1675                1680

Leu His  Val Leu Pro Leu Tyr  Arg Leu Ala Asp Thr  Asp Glu Phe
    1685                1690                1695

Gly Ser  Val Glu Gly Met Lys  Ala Lys Ile Lys Ser  Gly Ala Ile
    1700                1705                1710

Gln Val  Asn Gly Pro Thr Arg  Lys Arg Arg Leu Arg  Phe Thr Glu
    1715                1720                1725

Pro Val  Pro Arg Cys Gly Lys  Arg Ala Lys Met Lys  Gln Asn His
    1730                1735                1740

Asn Lys  Ser Gly Ser His Asn  Thr Lys Ser Phe Ser  Ser Ala Ser
    1745                1750                1755

Ser Thr  Ser His Leu Val Lys  Asp Glu Ser Thr Asp  Phe Cys Pro
    1760                1765                1770

Leu Gln  Ala Ser Ser Ala Glu  Thr Ser Thr Cys Thr  Tyr Ser Lys
    1775                1780                1785

Thr Ala  Ser Gly Gly Phe Ala  Glu Thr Ser Ser Ile  Leu His Cys
    1790                1795                1800

Thr Met  Pro Ser Gly Ala His  Ser Gly Ala Asn Ala  Ala Ala Gly
    1805                1810                1815

Glu Cys  Thr Gly Thr Val Gln  Pro Ala Glu Val Ala  Ala His Pro
    1820                1825                1830

His Gln  Ser Leu Pro Thr Ala  Asp Ser Pro Val His  Ala Glu Pro
    1835                1840                1845

Leu Thr  Ser Pro Ser Glu Gln  Leu Thr Ser Asn Gln  Ser Asn Gln
    1850                1855                1860

Gln Leu  Pro Leu Leu Ser Asn  Ser Gln Lys Leu Ala  Ser Cys Gln
    1865                1870                1875

Val Glu  Asp Glu Arg His Pro  Glu Ala Asp Glu Pro  Gln His Pro
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    1880                1885                1890

Glu Asp  Asp Asn Leu Pro Gln  Leu Asp Glu Phe Trp  Ser Asp Ser
    1895                1900                1905

Glu Glu  Ile Tyr Ala Asp Pro  Ser Phe Gly Gly Val  Ala Ile Ala
    1910                1915                1920

Pro Ile  His Gly Ser Val Leu  Ile Glu Cys Ala Arg  Lys Glu Leu
    1925                1930                1935

His Ala  Thr Thr Ser Leu Arg  Ser Pro Lys Arg Gly  Val Pro Phe
    1940                1945                1950

Arg Val  Ser Leu Val Phe Tyr  Gln His Lys Ser Leu  Asn Lys Pro
    1955                1960                1965

Asn His  Gly Phe Asp Ile Asn  Lys Ile Lys Cys Lys  Cys Lys Lys
    1970                1975                1980

Val Thr  Lys Lys Lys Pro Ala  Asp Arg Glu Cys Pro  Asp Val Ser
    1985                1990                1995

Pro Glu  Ala Asn Leu Ser His  Gln Ile Pro Ser Arg  Val Ala Ser
    2000                2005                2010

Thr Leu  Thr Arg Asp Asn Val  Val Thr Val Ser Pro  Tyr Ser Leu
    2015                2020                2025

Thr His  Val Ala Gly Pro Tyr  Asn Arg Trp Val
    2030                2035


<210> SEQ ID NO 13
<211> LENGTH: 1912
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 13

Met Glu Gln Asp Arg Thr Thr His Ala Glu Gly Thr Arg Leu Ser Pro
1               5                   10                  15

Phe Leu Ile Ala Pro Pro Ser Pro Ile Ser His Thr Glu Pro Leu Ala
            20                  25                  30

Val Lys Leu Gln Asn Gly Ser Pro Leu Ala Glu Arg Pro His Pro Glu
        35                  40                  45

Val Asn Gly Asp Thr Lys Trp Gln Ser Ser Gln Ser Cys Tyr Gly Ile
    50                  55                  60

Ser His Met Lys Gly Ser Gln Ser Ser His Glu Ser Pro His Glu Asp
65                  70                  75                  80

Arg Gly Tyr Ser Arg Cys Leu Gln Asn Gly Gly Ile Lys Arg Thr Val
                85                  90                  95

Ser Glu Pro Ser Leu Ser Gly Leu His Pro Asn Lys Ile Leu Lys Leu
            100                 105                 110

Asp Gln Lys Ala Lys Gly Glu Ser Asn Ile Phe Glu Glu Ser Gln Glu
        115                 120                 125

Arg Asn His Gly Lys Ser Ser Arg Gln Pro Asn Val Ser Gly Leu Ser
    130                 135                 140

Asp Asn Gly Glu Pro Val Thr Ser Thr Thr Gln Glu Ser Ser Gly Ala
145                 150                 155                 160

Asp Ala Phe Pro Thr Arg Asn Tyr Asn Gly Val Glu Ile Gln Val Leu
                165                 170                 175

Asn Glu Gln Glu Gly Glu Lys Gly Arg Ser Val Thr Leu Leu Lys Asn
            180                 185                 190

Lys Ile Val Leu Met Pro Asn Gly Ala Thr Val Ser Ala His Ser Glu
        195                 200                 205
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Glu Asn Thr Arg Gly Glu Leu Glu Lys Thr Gln Cys Tyr Pro Asp
    210                 215                 220

Cys Val Ser Ile Ala Val Gln Ser Thr Ala Ser His Val Asn Thr Pro
225                 230                 235                 240

Ser Ser Gln Ala Ala Ile Glu Leu Ser His Glu Ile Pro Gln Pro Ser
                245                 250                 255

Leu Thr Ser Ala Gln Ile Asn Phe Ser Gln Thr Ser Ser Leu Gln Leu
            260                 265                 270

Pro Pro Glu Pro Ala Ala Met Val Thr Lys Ala Cys Asp Ala Asp Asn
        275                 280                 285

Ala Ser Lys Pro Ala Ile Val Pro Gly Thr Cys Pro Phe Gln Lys Ala
    290                 295                 300

Glu His Gln Gln Lys Ser Ala Leu Asp Ile Gly Pro Ser Arg Ala Glu
305                 310                 315                 320

Asn Lys Thr Ile Gln Gly Ser Met Glu Leu Phe Ala Glu Glu Tyr Tyr
                325                 330                 335

Pro Ser Ser Asp Arg Asn Leu Gln Ala Ser His Gly Ser Ser Glu Gln
            340                 345                 350

Tyr Ser Lys Gln Lys Glu Thr Asn Gly Ala Tyr Phe Arg Gln Ser Ser
        355                 360                 365

Lys Phe Pro Lys Asp Ser Ile Ser Pro Thr Thr Val Thr Pro Pro Ser
    370                 375                 380

Gln Ser Leu Leu Ala Pro Arg Leu Val Leu Gln Pro Pro Leu Glu Gly
385                 390                 395                 400

Lys Gly Ala Leu Asn Asp Val Ala Leu Glu Glu His His Asp Tyr Pro
                405                 410                 415

Asn Arg Ser Asn Arg Thr Leu Leu Arg Glu Gly Lys Ile Asp His Gln
            420                 425                 430

Pro Lys Thr Ser Ser Ser Gln Ser Leu Asn Pro Ser Val His Thr Pro
        435                 440                 445

Asn Pro Pro Leu Met Leu Pro Glu Gln His Gln Asn Asp Cys Gly Ser
    450                 455                 460

Pro Ser Pro Glu Lys Ser Arg Lys Met Ser Glu Tyr Leu Met Tyr Tyr
465                 470                 475                 480

Leu Pro Asn His Gly His Ser Gly Gly Leu Gln Glu His Ser Gln Tyr
                485                 490                 495

Leu Met Gly His Arg Glu Gln Glu Ile Pro Lys Asp Ala Asn Gly Lys
            500                 505                 510

Gln Thr Gln Gly Ser Val Gln Ala Ala Pro Gly Trp Ile Glu Leu Lys
        515                 520                 525

Ala Pro Asn Leu His Glu Ala Leu His Gln Thr Lys Arg Lys Asp Ile
    530                 535                 540

Ser Leu His Ser Val Leu His Ser Gln Thr Gly Pro Val Asn Gln Met
545                 550                 555                 560

Ser Ser Lys Gln Ser Thr Gly Asn Val Asn Met Pro Gly Gly Phe Gln
                565                 570                 575

Arg Leu Pro Tyr Leu Gln Lys Thr Ala Gln Pro Glu Gln Lys Ala Gln
            580                 585                 590

Met Tyr Gln Val Gln Val Asn Gln Gly Pro Ser Pro Gly Met Gly Asp
        595                 600                 605

Gln His Leu Gln Phe Gln Lys Ala Leu Tyr Gln Glu Cys Ile Pro Arg
    610                 615                 620

Thr Asp Pro Ser Ser Glu Ala His Pro Gln Ala Pro Ser Val Pro Gln
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625 630 635 640

Tyr His Phe Gln Gln Arg Val Asn Pro Ser Ser Asp Lys His Leu Ser
645 650 655

Gln Gln Ala Thr Glu Thr Gln Arg Leu Ser Gly Phe Leu Gln His Thr
660 665 670

Pro Gln Thr Gln Ala Ser Gln Thr Pro Ala Ser Gln Asn Ser Asn Phe
675 680 685

Pro Gln Ile Cys Gln Gln Gln Gln Gln Gln Leu Gln Arg Lys Asn
690 695 700

Lys Glu Gln Met Pro Gln Thr Phe Ser His Leu Gln Gly Ser Asn Asp
705 710 715 720

Lys Gln Arg Glu Gly Ser Cys Phe Gly Gln Ile Lys Val Glu Glu Ser
725 730 735

Phe Cys Val Gly Asn Gln Tyr Ser Lys Ser Ser Asn Phe Gln Thr His
740 745 750

Asn Asn Thr Gln Gly Gly Leu Glu Gln Val Gln Asn Ile Asn Lys Asn
755 760 765

Phe Pro Tyr Ser Lys Ile Leu Thr Pro Asn Ser Ser Asn Leu Gln Ile
770 775 780

Leu Pro Ser Asn Asp Thr His Pro Ala Cys Glu Arg Glu Gln Ala Leu
785 790 795 800

His Pro Val Gly Ser Lys Thr Ser Asn Leu Gln Asn Met Gln Tyr Phe
805 810 815

Pro Asn Asn Val Thr Pro Asn Gln Asp Val His Arg Cys Phe Gln Glu
820 825 830

Gln Ala Gln Lys Pro Gln Gln Ala Ser Ser Leu Gln Gly Leu Lys Asp
835 840 845

Arg Ser Gln Gly Glu Ser Pro Ala Pro Pro Ala Glu Ala Ala Gln Gln
850 855 860

Arg Tyr Leu Val His Asn Glu Ala Lys Ala Leu Pro Val Pro Glu Gln
865 870 875 880

Gly Gly Ser Gln Thr Gln Thr Pro Pro Gln Lys Asp Thr Gln Lys His
885 890 895

Ala Ala Leu Arg Trp Leu Leu Leu Gln Lys Gln Glu Gln Gln Gln Thr
900 905 910

Gln Gln Ser Gln Pro Gly His Asn Gln Met Leu Arg Pro Ile Lys Thr
915 920 925

Glu Pro Val Ser Lys Pro Ser Ser Tyr Arg Tyr Pro Leu Ser Pro Pro
930 935 940

Gln Glu Asn Met Ser Ser Arg Ile Lys Gln Glu Ile Ser Ser Pro Ser
945 950 955 960

Arg Asp Asn Gly Gln Pro Lys Ser Ile Ile Glu Thr Met Glu Gln His
965 970 975

Leu Lys Gln Phe Gln Leu Lys Ser Leu Cys Asp Tyr Lys Ala Leu Thr
980 985 990

Leu Lys Ser Gln Lys His Val Lys Val Pro Thr Asp Ile Gln Ala Ala
995 1000 1005

Glu Ser Glu Asn His Ala Arg Ala Ala Glu Pro Gln Ala Thr Lys
1010 1015 1020

Ser Thr Asp Cys Ser Val Leu Asp Asp Val Ser Glu Ser Asp Thr
1025 1030 1035

Pro Gly Glu Gln Ser Gln Asn Gly Lys Cys Glu Gly Cys Asn Pro
1040 1045 1050

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Asp Lys  Asp Glu Ala Pro Tyr  Tyr Thr His Leu Gly  Ala Gly Pro
    1055                 1060                 1065

Asp Val  Ala Ala Ile Arg Thr  Leu Met Glu Glu Arg  Tyr Gly Glu
    1070                 1075                 1080

Lys Gly  Lys Ala Ile Arg Ile  Glu Lys Val Ile Tyr  Thr Gly Lys
    1085                 1090                 1095

Glu Gly  Lys Ser Ser Gln Gly  Cys Pro Ile Ala Lys  Trp Val Tyr
    1100                 1105                 1110

Arg Arg  Ser Ser Glu Glu Glu  Lys Leu Leu Cys Leu  Val Arg Val
    1115                 1120                 1125

Arg Pro  Asn His Thr Cys Glu  Thr Ala Val Met Val  Ile Ala Ile
    1130                 1135                 1140

Met Leu  Trp Asp Gly Ile Pro  Lys Leu Leu Ala Ser  Glu Leu Tyr
    1145                 1150                 1155

Ser Glu  Leu Thr Asp Ile Leu  Gly Lys Cys Gly Ile  Cys Thr Asn
    1160                 1165                 1170

Arg Arg  Cys Ser Gln Asn Glu  Thr Arg Asn Cys Cys  Cys Gln Gly
    1175                 1180                 1185

Glu Asn  Pro Glu Thr Cys Gly  Ala Ser Phe Ser Phe  Gly Cys Ser
    1190                 1195                 1200

Trp Ser  Met Tyr Tyr Asn Gly  Cys Lys Phe Ala Arg  Ser Lys Lys
    1205                 1210                 1215

Pro Arg  Lys Phe Arg Leu His  Gly Ala Glu Pro Lys  Glu Glu Glu
    1220                 1225                 1230

Arg Leu  Gly Ser His Leu Gln  Asn Leu Ala Thr Val  Ile Ala Pro
    1235                 1240                 1245

Ile Tyr  Lys Lys Leu Ala Pro  Asp Ala Tyr Asn Asn  Gln Val Glu
    1250                 1255                 1260

Phe Glu  His Gln Ala Pro Asp  Cys Cys Leu Gly Leu  Lys Glu Gly
    1265                 1270                 1275

Arg Pro  Phe Ser Gly Val Thr  Ala Cys Leu Asp Phe  Ser Ala His
    1280                 1285                 1290

Ser His  Arg Asp Gln Gln Asn  Met Pro Asn Gly Ser  Thr Val Val
    1295                 1300                 1305

Val Thr  Leu Asn Arg Glu Asp  Asn Arg Glu Val Gly  Ala Lys Pro
    1310                 1315                 1320

Glu Asp  Glu Gln Phe His Val  Leu Pro Met Tyr Ile  Ile Ala Pro
    1325                 1330                 1335

Glu Asp  Glu Phe Gly Ser Thr  Glu Gly Gln Glu Lys  Lys Ile Arg
    1340                 1345                 1350

Met Gly  Ser Ile Glu Val Leu  Gln Ser Phe Arg Arg  Arg Arg Val
    1355                 1360                 1365

Ile Arg  Ile Gly Glu Leu Pro  Lys Ser Cys Lys Lys  Lys Ala Glu
    1370                 1375                 1380

Pro Lys  Lys Ala Lys Thr Lys  Lys Ala Ala Arg Lys  Arg Ser Ser
    1385                 1390                 1395

Leu Glu  Asn Cys Ser Ser Arg  Thr Glu Lys Gly Lys  Ser Ser Ser
    1400                 1405                 1410

His Thr  Lys Leu Met Glu Asn  Ala Ser His Met Lys  Gln Met Thr
    1415                 1420                 1425

Ala Gln  Pro Gln Leu Ser Gly  Pro Val Ile Arg Gln  Pro Pro Thr
    1430                 1435                 1440
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Leu Gln  Arg His Leu Gln Gln  Gly Gln Arg Pro Gln  Gln Pro Gln
    1445                 1450                 1455

Pro Pro  Gln Pro Gln Pro Gln  Thr Thr Pro Gln Pro  Gln Pro Gln
    1460                 1465                 1470

Pro Gln  His Ile Met Pro Gly  Asn Ser Gln Ser Val  Gly Ser His
    1475                 1480                 1485

Cys Ser  Gly Ser Thr Ser Val  Tyr Thr Arg Gln Pro  Thr Pro His
    1490                 1495                 1500

Ser Pro  Tyr Pro Ser Ser Ala  His Thr Ser Asp Ile  Tyr Gly Asp
    1505                 1510                 1515

Thr Asn  His Val Asn Phe Tyr  Pro Thr Ser Ser His  Ala Ser Gly
    1520                 1525                 1530

Ser Tyr  Leu Asn Pro Ser Asn  Tyr Met Asn Pro Tyr  Leu Gly Leu
    1535                 1540                 1545

Leu Asn  Gln Asn Asn Gln Tyr  Ala Pro Phe Pro Tyr  Asn Gly Ser
    1550                 1555                 1560

Val Pro  Val Asp Asn Gly Ser  Pro Phe Leu Gly Ser  Tyr Ser Pro
    1565                 1570                 1575

Gln Ala  Gln Ser Arg Asp Leu  His Arg Tyr Pro Asn  Gln Asp His
    1580                 1585                 1590

Leu Thr  Asn Gln Asn Leu Pro  Pro Ile His Thr Leu  His Gln Gln
    1595                 1600                 1605

Thr Phe  Gly Asp Ser Pro Ser  Lys Tyr Leu Ser Tyr  Gly Asn Gln
    1610                 1615                 1620

Asn Met  Gln Arg Asp Ala Phe  Thr Thr Asn Ser Thr  Leu Lys Pro
    1625                 1630                 1635

Asn Val  His His Leu Ala Thr  Phe Ser Pro Tyr Pro  Thr Pro Lys
    1640                 1645                 1650

Met Asp  Ser His Phe Met Gly  Ala Ala Ser Arg Ser  Pro Tyr Ser
    1655                 1660                 1665

His Pro  His Thr Asp Tyr Lys  Thr Ser Glu His His  Leu Pro Ser
    1670                 1675                 1680

His Thr  Ile Tyr Ser Tyr Thr  Ala Ala Ala Ser Gly  Ser Ser Ser
    1685                 1690                 1695

Ser His  Ala Phe His Asn Lys  Glu Asn Asp Asn Ile  Ala Asn Gly
    1700                 1705                 1710

Leu Ser  Arg Val Leu Pro Gly  Phe Asn His Asp Arg  Thr Ala Ser
    1715                 1720                 1725

Ala Gln  Glu Leu Leu Tyr Ser  Leu Thr Gly Ser Ser  Gln Glu Lys
    1730                 1735                 1740

Gln Pro  Glu Val Ser Gly Gln  Asp Ala Ala Ala Val  Gln Glu Ile
    1745                 1750                 1755

Glu Tyr  Trp Ser Asp Ser Glu  His Asn Phe Gln Asp  Pro Cys Ile
    1760                 1765                 1770

Gly Gly  Val Ala Ile Ala Pro  Thr His Gly Ser Ile  Leu Ile Glu
    1775                 1780                 1785

Cys Ala  Lys Cys Glu Val His  Ala Thr Thr Lys Val  Asn Asp Pro
    1790                 1795                 1800

Asp Arg  Asn His Pro Thr Arg  Ile Ser Leu Val Leu  Tyr Arg His
    1805                 1810                 1815

Lys Asn  Leu Phe Leu Pro Lys  His Cys Leu Ala Leu  Trp Glu Ala
    1820                 1825                 1830

Lys Met  Ala Glu Lys Ala Arg  Lys Glu Glu Glu Cys  Gly Lys Asn
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    1835                1840                1845

Gly Ser  Asp His Val Ser Gln  Lys Asn His Gly Lys  Gln Glu Lys
    1850                1855                1860

Arg Glu  Pro Thr Gly Pro Gln  Glu Pro Ser Tyr Leu  Arg Phe Ile
    1865                1870                1875

Gln Ser  Leu Ala Glu Asn Thr  Gly Ser Val Thr Thr  Asp Ser Thr
    1880                1885                1890

Val Thr  Thr Ser Pro Tyr Ala  Phe Thr Gln Val Thr  Gly Pro Tyr
    1895                1900                1905

Asn Thr  Phe Val
    1910


<210> SEQ ID NO 14
<211> LENGTH: 1713
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 14

Met Phe Leu Pro Glu Thr Pro Gln Gln Tyr Ala Val Glu Ile Asn Ala
1               5                   10                  15

Arg Glu Gly Thr Gly Pro Trp Ala Gln Gly Ala Thr Val Lys Thr Gly
            20                  25                  30

Ser Glu Leu Ser Pro Val Asp Gly Pro Val Pro Gly Gln Met Asp Ser
        35                  40                  45

Gly Pro Val Tyr His Gly Asp Ser Arg Gln Leu Ser Thr Ser Gly Ala
    50                  55                  60

Pro Val Asn Gly Ala Arg Glu Pro Ala Gly Pro Gly Leu Leu Gly Ala
65                  70                  75                  80

Ala Gly Pro Trp Arg Val Asp Gln Lys Pro Asp Trp Glu Ala Ala Ser
                85                  90                  95

Gly Pro Thr His Ala Ala Arg Leu Glu Asp Ala His Asp Leu Val Ala
            100                 105                 110

Phe Ser Ala Val Ala Glu Ala Val Ser Ser Tyr Gly Ala Leu Ser Thr
        115                 120                 125

Arg Leu Tyr Glu Thr Phe Asn Arg Glu Met Ser Arg Glu Ala Gly Ser
    130                 135                 140

Asn Gly Arg Gly Pro Arg Pro Glu Ser Cys Ser Glu Gly Ser Glu Asp
145                 150                 155                 160

Leu Asp Thr Leu Gln Thr Ala Leu Ala Leu Ala Arg His Gly Met Lys
                165                 170                 175

Pro Pro Asn Cys Thr Cys Asp Gly Pro Glu Cys Pro Asp Phe Leu Glu
            180                 185                 190

Trp Leu Glu Gly Lys Ile Lys Ser Met Ala Met Glu Gly Gly Gln Gly
        195                 200                 205

Arg Pro Arg Leu Pro Gly Ala Leu Pro Pro Ser Glu Ala Gly Leu Pro
    210                 215                 220

Ala Pro Ser Thr Arg Pro Pro Leu Leu Ser Ser Glu Val Pro Gln Val
225                 230                 235                 240

Pro Pro Leu Glu Gly Leu Pro Leu Ser Gln Ser Ala Leu Ser Ile Ala
                245                 250                 255

Lys Glu Lys Asn Ile Ser Leu Gln Thr Ala Ile Ala Ile Glu Ala Leu
            260                 265                 270

Thr Gln Leu Ser Ser Ala Leu Pro Gln Pro Ser His Ser Thr Ser Gln
        275                 280                 285
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Ala Ser Cys Pro Leu Pro Glu Ala Leu Ser Pro Ser Ala Pro Phe Arg
    290                 295                 300

Ser Pro Gln Ser Tyr Leu Arg Ala Pro Ser Trp Pro Val Val Pro Pro
305                 310                 315                 320

Glu Glu His Pro Ser Phe Ala Pro Asp Ser Pro Ala Phe Pro Pro Ala
                325                 330                 335

Thr Pro Arg Pro Glu Phe Ser Glu Ala Trp Gly Thr Asp Thr Pro Pro
            340                 345                 350

Ala Thr Pro Arg Asn Ser Trp Pro Val Pro Arg Pro Ser Pro Asp Pro
        355                 360                 365

Met Ala Glu Leu Glu Gln Leu Leu Gly Ser Ala Ser Asp Tyr Ile Gln
    370                 375                 380

Ser Val Phe Lys Arg Pro Glu Ala Leu Pro Thr Lys Pro Lys Val Lys
385                 390                 395                 400

Val Glu Ala Pro Ser Ser Ser Pro Ala Pro Val Pro Ser Pro Ile Ser
                405                 410                 415

Gln Arg Glu Ala Pro Leu Leu Ser Ser Glu Pro Asp Thr His Gln Lys
            420                 425                 430

Ala Gln Thr Ala Leu Gln Gln His Leu His His Lys Arg Asn Leu Phe
        435                 440                 445

Leu Glu Gln Ala Gln Asp Ala Ser Phe Pro Thr Ser Thr Glu Pro Gln
    450                 455                 460

Ala Pro Gly Trp Trp Ala Pro Pro Gly Ser Pro Ala Pro Arg Pro Pro
465                 470                 475                 480

Asp Lys Pro Pro Lys Glu Lys Lys Lys Lys Pro Pro Thr Pro Ala Gly
                485                 490                 495

Gly Pro Val Gly Ala Glu Lys Thr Thr Pro Gly Ile Lys Thr Ser Val
            500                 505                 510

Arg Lys Pro Ile Gln Ile Lys Lys Ser Arg Ser Arg Asp Met Gln Pro
        515                 520                 525

Leu Phe Leu Pro Val Arg Gln Ile Val Leu Glu Gly Leu Lys Pro Gln
    530                 535                 540

Ala Ser Glu Gly Gln Ala Pro Leu Pro Ala Gln Leu Ser Val Pro Pro
545                 550                 555                 560

Pro Ala Ser Gln Gly Ala Ala Ser Gln Ser Cys Ala Thr Pro Leu Thr
                565                 570                 575

Pro Glu Pro Ser Leu Ala Leu Phe Ala Pro Ser Pro Ser Gly Asp Ser
            580                 585                 590

Leu Leu Pro Pro Thr Gln Glu Met Arg Ser Pro Ser Pro Met Val Ala
        595                 600                 605

Leu Gln Ser Gly Ser Thr Gly Gly Pro Leu Pro Pro Ala Asp Asp Lys
    610                 615                 620

Leu Glu Glu Leu Ile Arg Gln Phe Glu Ala Glu Phe Gly Asp Ser Phe
625                 630                 635                 640

Gly Leu Pro Gly Pro Pro Ser Val Pro Ile Gln Glu Pro Glu Asn Gln
                645                 650                 655

Ser Thr Cys Leu Pro Ala Pro Glu Ser Pro Phe Ala Thr Arg Ser Pro
            660                 665                 670

Lys Lys Ile Lys Ile Glu Ser Ser Gly Ala Val Thr Val Leu Ser Thr
        675                 680                 685

Thr Cys Phe His Ser Glu Glu Gly Gly Gln Glu Ala Thr Pro Thr Lys
    690                 695                 700

Ala Glu Asn Pro Leu Thr Pro Thr Leu Ser Gly Phe Leu Glu Ser Pro
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705                 710                 715                 720

Leu Lys Tyr Leu Asp Thr Pro Thr Lys Ser Leu Leu Asp Thr Pro Ala
                725                 730                 735

Lys Lys Ala Gln Ser Glu Phe Pro Thr Cys Asp Cys Val Glu Gln Ile
            740                 745                 750

Val Glu Lys Asp Glu Gly Pro Tyr Tyr Thr His Leu Gly Ser Gly Pro
        755                 760                 765

Thr Val Ala Ser Ile Arg Glu Leu Met Glu Asp Arg Tyr Gly Glu Lys
    770                 775                 780

Gly Lys Ala Ile Arg Ile Glu Lys Val Ile Tyr Thr Gly Lys Glu Gly
785                 790                 795                 800

Lys Ser Ser Arg Gly Cys Pro Ile Ala Lys Trp Val Ile Arg Arg His
                805                 810                 815

Thr Leu Glu Glu Lys Leu Leu Cys Leu Val Arg His Arg Ala Gly His
            820                 825                 830

His Cys Gln Asn Ala Val Ile Val Ile Leu Ile Leu Ala Trp Glu Gly
        835                 840                 845

Ile Pro Arg Ser Leu Gly Asp Thr Leu Tyr Gln Glu Leu Thr Asp Thr
    850                 855                 860

Leu Arg Lys Tyr Gly Asn Pro Thr Ser Arg Arg Cys Gly Leu Asn Asp
865                 870                 875                 880

Asp Arg Thr Cys Ala Cys Gln Gly Lys Asp Pro Asn Thr Cys Gly Ala
                885                 890                 895

Ser Phe Ser Phe Gly Cys Ser Trp Ser Met Tyr Phe Asn Gly Cys Lys
            900                 905                 910

Tyr Ala Arg Ser Lys Thr Pro Arg Lys Phe Arg Leu Thr Gly Asp Asn
        915                 920                 925

Pro Lys Glu Glu Glu Val Leu Arg Asn Ser Phe Gln Asp Leu Ala Thr
    930                 935                 940

Glu Val Ala Pro Leu Tyr Lys Arg Leu Ala Pro Gln Ala Tyr Gln Asn
945                 950                 955                 960

Gln Val Thr Asn Glu Asp Val Ala Ile Asp Cys Arg Leu Gly Leu Lys
                965                 970                 975

Glu Gly Arg Pro Phe Ser Gly Val Thr Ala Cys Met Asp Phe Cys Ala
            980                 985                 990

His Ala His Lys Asp Gln His Asn  Leu Tyr Asn Gly Cys  Thr Val Val
        995                 1000                1005

Cys Thr  Leu Thr Lys Glu Asp  Asn Arg Cys Val Gly  Gln Ile Pro
    1010                1015                1020

Glu Asp  Glu Gln Leu His Val  Leu Pro Leu Tyr Lys  Met Ala Ser
    1025                1030                1035

Thr Asp  Glu Phe Gly Ser Glu  Glu Asn Gln Asn Ala  Lys Val Ser
    1040                1045                1050

Ser Gly  Ala Ile Gln Val Leu  Thr Ala Phe Pro Arg  Glu Val Arg
    1055                1060                1065

Arg Leu  Pro Glu Pro Ala Lys  Ser Cys Arg Gln Arg  Gln Leu Glu
    1070                1075                1080

Ala Arg  Lys Ala Ala Ala Glu  Lys Lys Lys Leu Gln  Lys Glu Lys
    1085                1090                1095

Leu Ser  Thr Pro Glu Lys Ile  Lys Gln Glu Ala Leu  Glu Leu Ala
    1100                1105                1110

Gly Val  Thr Thr Asp Pro Gly  Leu Ser Leu Lys Gly  Gly Leu Ser
    1115                1120                1125
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Gln Gln  Ser Leu Lys Pro Ser  Leu Lys Val Glu Pro  Gln Asn His
    1130                 1135                 1140

Phe Ser  Ser Phe Lys Tyr Ser  Gly Asn Ala Val Val  Glu Ser Tyr
    1145                 1150                 1155

Ser Val  Leu Gly Ser Cys Arg  Pro Ser Asp Pro Tyr  Ser Met Ser
    1160                 1165                 1170

Ser Val  Tyr Ser Tyr His Ser  Arg Tyr Ala Gln Pro  Gly Leu Ala
    1175                 1180                 1185

Ser Val  Asn Gly Phe His Ser  Lys Tyr Thr Leu Pro  Ser Phe Gly
    1190                 1195                 1200

Tyr Tyr  Gly Phe Pro Ser Ser  Asn Pro Val Phe Pro  Ser Gln Phe
    1205                 1210                 1215

Leu Gly  Pro Ser Ala Trp Gly  His Gly Gly Ser Gly  Gly Ser Phe
    1220                 1225                 1230

Glu Lys  Lys Pro Asp Leu His  Ala Leu His Asn Ser  Leu Asn Pro
    1235                 1240                 1245

Ala Tyr  Gly Gly Ala Glu Phe  Ala Glu Leu Pro Gly  Gln Ala Val
    1250                 1255                 1260

Ala Thr  Asp Asn His His Pro  Ile Pro His His Gln  Gln Pro Ala
    1265                 1270                 1275

Tyr Pro  Gly Pro Lys Glu Tyr  Leu Leu Pro Lys Val  Pro Gln Leu
    1280                 1285                 1290

His Pro  Ala Ser Arg Asp Pro  Ser Pro Phe Ala Gln  Ser Ser Ser
    1295                 1300                 1305

Cys Tyr  Asn Arg Ser Ile Lys  Gln Glu Pro Ile Asp  Pro Leu Thr
    1310                 1315                 1320

Gln Ala  Glu Ser Ile Pro Arg  Asp Ser Ala Lys Met  Ser Arg Thr
    1325                 1330                 1335

Pro Leu  Pro Glu Ala Ser Gln  Asn Gly Gly Pro Ser  His Leu Trp
    1340                 1345                 1350

Gly Gln  Tyr Ser Gly Gly Pro  Ser Met Ser Pro Lys  Arg Thr Asn
    1355                 1360                 1365

Ser Val  Gly Gly Asn Trp Gly  Val Phe Pro Pro Gly  Glu Ser Pro
    1370                 1375                 1380

Thr Ile  Val Pro Asp Lys Leu  Asn Ser Phe Gly Ala  Ser Cys Leu
    1385                 1390                 1395

Thr Pro  Ser His Phe Pro Glu  Ser Gln Trp Gly Leu  Phe Thr Gly
    1400                 1405                 1410

Glu Gly  Gln Gln Ser Ala Pro  His Ala Gly Ala Arg  Leu Arg Gly
    1415                 1420                 1425

Lys Pro  Trp Ser Pro Cys Lys  Phe Gly Asn Gly Thr  Ser Ala Leu
    1430                 1435                 1440

Thr Gly  Pro Ser Leu Thr Glu  Lys Pro Trp Gly Met  Gly Thr Gly
    1445                 1450                 1455

Asp Phe  Asn Pro Ala Leu Lys  Gly Gly Pro Gly Phe  Gln Asp Lys
    1460                 1465                 1470

Leu Trp  Asn Pro Val Lys Val  Glu Glu Gly Arg Ile  Pro Thr Pro
    1475                 1480                 1485

Gly Ala  Asn Pro Leu Asp Lys  Ala Trp Gln Ala Phe  Gly Met Pro
    1490                 1495                 1500

Leu Ser  Ser Asn Glu Lys Leu  Phe Gly Ala Leu Lys  Ser Glu Glu
    1505                 1510                 1515
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Lys Leu  Trp Asp Pro Phe Ser  Leu Glu Glu Gly Thr  Ala Glu Glu
    1520                 1525                 1530

Pro Pro  Ser Lys Gly Val Val  Lys Glu Glu Lys Ser  Gly Pro Thr
    1535                 1540                 1545

Val Glu  Glu Asp Glu Glu Glu  Leu Trp Ser Asp Ser  Glu His Asn
    1550                 1555                 1560

Phe Leu  Asp Glu Asn Ile Gly  Gly Val Ala Val Ala  Pro Ala His
    1565                 1570                 1575

Cys Ser  Ile Leu Ile Glu Cys  Ala Arg Arg Glu Leu  His Ala Thr
    1580                 1585                 1590

Thr Pro  Leu Lys Lys Pro Asn  Arg Cys His Pro Thr  Arg Ile Ser
    1595                 1600                 1605

Leu Val  Phe Tyr Gln His Lys  Asn Leu Asn Gln Pro  Asn His Gly
    1610                 1615                 1620

Leu Ala  Leu Trp Glu Ala Lys  Met Lys Gln Leu Ala  Glu Arg Ala
    1625                 1630                 1635

Arg Gln  Arg Gln Glu Glu Ala  Ala Arg Leu Gly Leu  Gly Gln Gln
    1640                 1645                 1650

Glu Ala  Lys Leu Tyr Gly Lys  Lys Arg Lys Trp Gly  Gly Ala Met
    1655                 1660                 1665

Val Ala  Glu Pro Gln His Lys  Glu Lys Lys Gly Ala  Ile Pro Thr
    1670                 1675                 1680

Arg Gln  Ala Leu Ala Met Pro  Thr Asp Ser Ala Val  Thr Val Ser
    1685                 1690                 1695

Ser Tyr  Ala Tyr Thr Lys Val  Thr Gly Pro Tyr Ser  Arg Trp Ile
    1700                 1705                 1710
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What is claimed is:

1. A composition comprising a reducing agent capable of converting 5-formylcytosine or 5-carboxycytosine to 5-hydroxymethylcytosine; T4 β-glucosyltransferase (BGT) and a UDP derivative selected from the group consisting of UDP-glucose and UDP-azidoglucose.

2. A composition according to claim **1**, further comprising a nucleic acid.

3. A kit comprising a reducing agent capable of converting 5-formylcytosine or 5-carboxycytosine to 5-hydroxymethylcytosine; T4 β-glucosyltransferase (BGT); and a UDP derivative selected from the group consisting of UDP-glucose and UDP-azidoglucose.

4. A kit according to claim **3**, further comprising UDP-glucosamine.

5. A kit according to claim **3**, further comprising a nucleic acid.

6. A kit according to any claim **3**, further comprising an oxidizing agent that is capable of oxidizing 5-methylcytosine to 5-hydroxymethylcytosine.

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